Chapter 4

Use of Multi-Nutrient Functional Peptide Complex «GRINIZATION» for treatment and prevention of viral infections

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1. Introduction

On the edge of XX–XXI centuries the further studies concerning the food problem's development, was registered beginning from the studying of the different nutrients as well as their influence on the level of common health. The population nutrition, especially which of children, is the determining factor in the national gene foundation preservation, health strengthening and the prophylactics of the number of diseases. The problem of the rational nutrition of various groups of the population now, as well as in the last century, is the great social and economical problem. The nutrition balance's discrepancies cause the fatal influence on the human health. The results of the wide epidemiological studies of the nutrition state of the children and the persons of the old age have shown that their nutrition structure is being characterized by serious disbalance. The social and economical damage caused by the diseases initiated by the nutrients' deficit is enormously significant. Beginning from the second half of the late century, the great majority of the population of the countries of the world suffer the negative changes in the nutrition caused by the technologic and genetic pollution of the nutrients as well as the use of the highly refined products and the decrease of the non-substitutional micro-nutrients' content.

The new components such as the trans-fatty acids, sugar substituents, odorants, taste batterers, colorants and consistency equalizers which were unknown to human organism and the enzyme systems of the later were adapted to these substances. The xenobiotics, oriented to the preservation time prolongation, taste and odor bettering, as well as the coloration of the products, cause the proteins and enzymes destruction. The contemporary human being, using certain nutrition product, couldn't be sure that his/her organism will receive all the needed substances in those combinations that were in nature before.

The last years, the humans using the nutrients, think more and more frequently not about their real usefulness but about the minimization of the harm to the organism that could be caused by their use. The nutrition of the modern human is characterized by the disbalance between the use of the great quantity of the products containing «pure» calories and the use of the palm oil and other fats. These products have the serious deficit of the vitamins, essential mineral substances, natural fats, anti-oxidants and non-substitutional amino acids (NSA). The permanent deficit of the required components causes the changes in the substances' exchange circle as well as the exhaustion of the inner resources of the proteins and calcium and, as a result, initiate the start of the "economy" mechanisms through the lowering the level of the substances' exchange level intensity, what leads

directly to the muscles' weakening, fatting, osteoporosis, immune-deficit state, the apoptosis of the weakened cells and oncogenesis.

Beginning from the fifties of the XX century, the influence of different substances on the biochemical and physiological processes in human organism is studied intensively as well as the possibilities of their use with the scope of prophylaxis and treatment. The influence of the majority of the micro- and ultramicro-elements such as: selenium, zinc, chromium, molybdenum, ubichinone, lycopene, zeaxantyne, luteine, quercetine, proantocyanides, rezveratrole, vegetable sterins, hondroprotectors and some fatty acids, was studied in details. About 700 food components are described and studied and their great majority is used in medical practice. In the parallel way to the food industry, the market of biologically active additives (BAA) is being cultivated as well as the products of the functional nutrition which are directed to support the ration of the contemporary human with the components absent in the regular food. The most wide spread is the use of the functional feeding products, BAA and special products for diet use is in Japan. The experience of the special feeding products use in Japan has more than 50 years and more than 90 % of the population certify the use of these products, in the U.S.A. — more than 20 % or 80 % of the population use them, in France and Germany — 60 %, and in Russia — about 3 %.

The main part of the functional feeding products, BAA and special products for diet feeding is the concentrates of the natural and of naturally identical BAA used for the enrichment of the ration with the specific substances or their complexes. Most of them contain the measured quantity of the components and, mainly, they are: the combination of the vitamins, minerals, some amino acids, fatty acids, phospholipids and vegetable anti-oxidants. The multi-nutrient functional-peptide complex which is composed by the two different complexes «Grinization Mix» and «Grinization Pro», differs significantly from the majority of the existing special products, due to the fact that it includes the natural extractions and extracts of the animal and vegetable origin, which contain all needed substances in their natural bounds and relations. During the use of «Grinization» preparations, the organism obtains once the balanced complex of all nutrients in the form of highly acceptable combinations obtained through special technology of preparation and processing.

1.1. Content of «Grin Mix» composition

- extract of active substances from natural kumiss (milk of a mare)
- spirulina, laminaria, cucumaria
- complex of omega fats of saltwater fish
- quail eggs powder
- oils from: buckthorn, flaxseed, wheat embryos, walachian nut, cedar, grape-stones

aqueous extracts of: brotherwort grass, pepper fruits, lovage (*Levisticum officinale*) roots, bottlebrush grass, nettle (*Urtica dioica*) leaves, plantain leaves, leaves and roots of parsley, aloe stem, onion, garlic, eleutherococcus root, flax seed, milk thistle (*Silybum*), burdock root, hop cones, bark of buckthorn

- vitamins: B_1, B_2, B_6, B_{12}, D_3, K_3
- copper complex
- grape-stones extract
- stevia, cherry syrup.

1.2. Content of «Grin Pro» composition

- complex protein-peptide composition of animal origin
- quail eggs powder
- artichoke powder, girasol powder
- lecithin, lactulose
- Saint-Mary-thistle (*Silybum marianum*) seeds; spirulina, lentil
- ascorbic acid.

1.3. The form of production and packing of MFPC «Grinization»

Form of production

- «Grin Mix» — a flask of 100 ml.
- «Grin Pro» — a flask of 70 capsules 400 mg each.
- «Grin Pro» — a flask of 50 g (powdered form)

1.4. The advantages of «Grinization» as compared to the existing metabolic compounds

Grinization complex has such advantages as:

- ***The natural origin*:** majority of existing metabolic compounds are synthesized or extracted and presented in the form of monopreparations;
- ***The absence of the influence on the digestion and metabolism of nutrients of the food ration:*** the digestion of the food nutrients depends on their correlation in the ration. The use of the given aminoacid, for example, arginin or taurin in the concentrations of treatment diminishes the digestion of other amino acids.
- ***The simplicity and security of the dosing*:** the vitamins and minerals which are included into the «Grinization» complex are represented by the natural animal and vegetable complexes

used in the ration of humans during the whole period of their evolution. Human don't digest separately the vitamins A, E, and omega-3 fat acids but the whole fatty complex which includes in the natural conditions all lipid substances (now almost the 700 food components are discovered and discussed, the carotinoids are more than 600 in number). Besides, they are digested in the organism along with amino acids, minerals, biflavanoids and other substances.

2. The Mechanism of Action of the Multi-Nutrients Functional-Peptide Complex «Grinization»

Multi-nutriental complex «Grinization» is created on the basis of the balanced feeding concept, the theory of the functional systems and ideas on the asymilation of the biologically active food components in the most acceptable form[1].

The existing forms of food preparation concerns, mainly, the use of high temperature, what results in irreversible denaturative changes of proteins, destruction or polymerization of the lipid components, destruction of the majority of vitamins and other unwanted changes. The introduction of the nutrients into human organism after the traditional ways of food preparation, their digestion is hindered both in digestive system and on the level of intercellular metabolism.

The producers of the multi-nutrient complex «Grinization» use the low temperature non-enzyme processing of the input product. The partial hydrolysis of the nucleoproteides, lipoproteids and proteins occurs. This fact allows to obtain the said compounds in the globular state under the conditions of their functional properties preservation but with the loss of the tissue specificity. Due to the use of such technology, all the nutrients enter the human organism in the bio-accessible forms.

Along with the use of the original technology, the content of «Grinization» complex includes the biologically active complexes of proteins and lipids, vitally significant macro- and micro-elements: calcium, potassium, sodium, magnesium, selenium, cobalt, molybdenum, ferrum, cuprum, zincum, iodium, which are supplied from various types of natural sources, including sea cucumber (cucumaria), spirulina, quail eggs, etc.

2.1. The Content of Amino Acids in Proteins in «Grinization» Complex

The food significance is the integral factor which reflects all the useful properties of the product, including the level of human organism supply with the most significant substances and energy. The biological significance of the protein depends on the presence and balance of the amino acids in its content. The content of amino acids in «Grinization» products is studied at the Institute of Biochemistry of National Academy of Sciences of Ukraine. The results obtained are presented in Table 1.

Table 1. The content of amino acids in proteins in «Grinization» complex, %

Amino acids	Grinization Mix	Grinization Pro
Non-substitutable amino acids	**40.99**	**43.59**
Valine	5.31	4.73
Isoleicine	4.79	4.47
Leicine	8.25	8.63
Lisine	5.84	8.61
Methionine	1.15	1.83
Treonine	5.08	5.77
Cistine	1.89	1.39
Tyrosine	3.97	3.65
Phenylalanine	4.71	4.51
Substitutable amino acids	**59.01**	**56.43**
Alanine	5.44	5.93
Argynine	5.28	5.93
Aspargine acid	21.70	9.97
Gystidine	1.93	3.61
Glutamine acid	12.57	17.83
Proline	2.06	3.15
Serine	5.95	5.15
Glycine	4.06	4.86

The biological significance of the protein is determined by the content of non-substitutable amino acids (NSA) and their balance. The results of the analysis have shown that the general quantity of NRA in the multi-nutrient complex is higher than in the ideal protein (according to the scale FAO/WHO). The substitutable amino acids which fulfill the functions of the predecessors during the protein and other biologically active substances' synthesis form 57–60 %. Such a type of amino acids content certifies the high biological significance of the product and allows to compensate in time the amino acids' deficit. Proteins are the main structural elements of each cell and realizes the following significant functions, e.g.:

- **Synthetic —** the synthesis of cell proteins, enzymes, haemoglobin as well;
- **Growth —** cells reparation and multiplication;
- **Catalytic** — enzyme assisted reactions, included the inner-cell ones;
- **Regulatory** — hormones' generation;
- **Structural** — collagen production for vessels' walls, skin, bones, teeth as well;
- **Muscular —** creation of miosine for muscules, myocardium, uterus;
- **Transport** — creation of haemoglobin, myoglobin, albumin and other transport proteins;
- **Buffer** — support the pH constancy of serum, spinal liquor and intestinal secrets;
- **Protective —** support of immune system efficacy;
- **Informative** — reverse connection of receptors' system to spinal and head brains.

2.2. Content of Fat Acids and Lipids in «Grinization» Complex

The high biological significance of the «Grinization» complex is being guaranteed by the presence of all needed fat acids, especially the poly-non-saturated ones (PNS) from the family of omega –3 and omega –6 in this complex (see Table 2, please).

Table 2. Content of fat acids and lipids in Multi-Nutrient-Functional Complex (MNFC) «Grinization» in % of the general quantity of fat acids

Acids Code	Grinization Mix	Grinization Pro	Adequate level of consumption
Saturated acids	**23.30**	**39.52**	**25**
Laurine 12:0	0.242	0.122	
Miristine 14:0	0.831	1.253	
Pentadecane 15:0	0.079	0.041	
Palmitine 16:0	15.56	21.62	
Margarine 17:0	0.787	0.631	
Stearine 18:0	5.809	15.86	
Mono-non-saturated acids	**28.93**	**33.97**	**30**
Miristoleine 14:1	0.089	0.155	
Pentadecene 15:1	0.165	0.075	
Palmitooleine 16:1	8.167	7.279	
Oleine 18:1	20.512	26.470	
Poly-non-saturated acids	**43.72**	**24.13**	**11**
Heptadecadiene 17:2	0.254	0.032	
Linole 18:2	23.530	18.815	
Linolene 18:3	13.710	2.113	
Arachine18:4	0.680	0.197	
Eikopentaene 20:3	1.758	0.086	
Arachidone 20:4	0.680	2.259	
Docosatetraene 22:4	0.151	0.205	
Docosapentaene 22:5	0.155	0.128	
Docosahexaene 22:6	2.809	0.301	
Omega-3 family (alpha-linolene, eicosapentaene, docosahexaene acids)	18.26	2.50	1
Omega-6 family (linole, gamma-linolene, conjugate linolene acids)	37.24	20.92	10

The biological significance of PNSA of various classes is caused by the fact that they perform significant functions as the structural blocks of the cell membranes and modulators of different biochemical processes. PNSA are, at the same time, the predecessors of regulatory mediators' creation, including: eicosanoids, prostaglandins, prostocyclins, thromboxanes and leicotrienes. The most significant is the correlation of the following classes of fat acids: saturated (SA), mono-non-saturated (MNA) and poly-non-saturated (PNSA) acids. In «Grinization Mix» complex this relation is equal to 23:28:43, while in «Grinization Pro» complex — 39:33:24, and in rational feeding 25:30:11. The question of the optimal value of certain fat acids in lipids of the food products is still under discussion. The most significant is, taking into account the biological efficacy of the lipids, the content of poly-non-saturated fat acids (PNFA).

The general quantity of PNFA in «Grinization» complexes exceeds sufficiently (by 2–4 times) their adequate level recommended for suggestion, what certifies the high biological activity of Grinization lipids.

The fats also have the plastic function as they are included into cell membranes and other cell structures as well. The central and peripheral nervous system are rich of the lipids. PNFA are included as well to the content of the cell membranes, and their most significant function is the synthesis of cell hormones — the prostoglandines. The properties of cell membranes as well as their reaction on the outer factors depend on the relation of PNFA concentration in cell components. The prostoglandines are created in human organism not only in tissues but also in thrombocytes (thromboxanes) and in leucocytes (leucotriens). The biological action of thrombocytes is extremely variant and depernds on PNFA type which are the basis for the fat acids creation.

The source of prostoglandines are the fat acids of omega-3 and omega-6 classes. Both these fat acids classes are the compulsory components of cell membranes but the ways of their metabolism and of action are sufficiently different. Such fat acids as linole and arachidone are the sources of prostocycline as well as of thromboxane. The fat acids of the omega-3 family with 20 carbon atoms and more displace the balance to the prostocycline synthesis. For example, the thromboxane A2 is synthesized from the arachidone acid what causes the thrombocytes aggregation and makes the vessels' walls thicker, the thromboxane A3 could be synthesized from eucosapentaene acid and this type of thromboxane doesn't have such an effect. Leucotriene B4 is the derivative of the arachidone acid and it belongs to the inflammation mediators, leucotriene B5, being synthesized from eucosapentaene acid demonstrates the anti-inflammatory effect.

2.3. Main Mechanisms of omega-3 Fat Acids' Action

Main mechanisms of omega-3 fat acids' action are:

- Eucosanoids synthesis regulation;
- The cell membranes phospholipids fat acids content modification;
- The influence on the inocyte lipid cycle and on the signal system;
- The influence on Ca^{2+}-chanels;
- The influence on the enzymes and cell receptors.

Omega-3 fat acids demonstrate the anti-inflammatory properties during the treatment of the inflammatory diseases of different ethiology due to the antagonistic properties towards the arachidone acid and its metabolits. Omega-3 fat acids hinder the synthesis of the inflammatory bound leucotriene B4, change the activity of proteinkinase C, influence the level of T- and B-lymphocytes, secretion of the lymphokines and cell proliferation. The recommended dose of omega-3 fat acids for healthy persons should be not less than 1.1 — 1.4 gram per day, including 1.1 gram — linolene and 0.3–0.4 grams of both eucosapentaene and docosahexaene acids.The «Grinization» complexes contain omega-3 fat acids in medicinal doses.

The hypothetic formula of ideal food fat was elaborated which, along with the content of the fat acids, were taken into account the atherogenic properties, the level of defence against the peroxide oxygenation of the lipids and the concentration of biologically active substances.

The Grinization complexes contain the high enough concentration of PNFA, including the omega-3 fat acids, what supports the high anti-atherogenic product's potential of the product. The Grinization PNFA are protected well from the free radical oxidation by the vitamins, by the minerals of the anti-oxidant direction and by the great number of vegetable anti-oxidants. Such a content of the Grinization complex guarantees the enhanced biological activity of the fat acids and more expressed regulatory impact.

2.4. The Contents of Vitamins in «Grinization» Complex

The vitamins are the non-substitutable participants of the feeding circle whose presence is necessary for the normal survival support as well as for the growth and reparation of the organism[2]. The significance of their presence in the food content is determined by the fact that these substances are the predecessors of different co-enzymes which participate in and control the metabolic processes.

Table 3. The contents of vitamins in «Grinization» complex, mg/100 grams

Vitamin	**Grinization Mix**	**Grinization Pro**
Vitamin B_1 (tiamine)	3.30±0.18	5.95 ± 0.18
Vitamin B_2 (riboflavine)	1.30 ± 0.1	0.68 ± 0.04
Vitamin B_5 (panthotene acid)	6.25 ± 0.40	4.00 ± 0.20
Vitamin B_6 (piridoxine)	tracks	0.13 ± 0.01
Vitamin B_9 (folic acid)	0.36 ± 0.01	0.19 ± 0.02
Vitamin B_{12} (cyanocobalamine)	0.07 ± 0.01	0.06 ± 0.005
Vitamin C (ascorbine acid)	24.60 ± 0.6	12.80 ± 0.50
Vitamkin E (tocoferol)	24.00 ± 0.005	17.50 ± 0.02
Vitamin A (retinole)	1.76 ± 0.03	0.17 ± 0.02
Carotinoids	45.96 ± 0.09	0.50 ± 0.10
Ubichinone Q_{10}	1.45± 0.4	7.37 ± 086
Ubichinone Q_9	0.91± 0.16	1.24 ± 0.23
Vitamin PP	14.40± 0.05	11.2±0.30

The presence of the vitamins B_2, C, PP and carotinoids in «Grinization Mix» complex in high enough concentration as well as the E and A vitamins guarantees the demonstration of the mainly anti-oxidant and anti-free-radicals biological actions. The «Grinization Pro» complex includes the vitamins B_1, B_2, B_9, PP, E, Q_{10} and carotinoids in high concentration as well. Taking into account the modern knowledge of their mechanisms of action and the biological role of these vitamins, it will be possible to explain the neurotropic effect, the non-specific immunity enhancement, the bio-energetic exchange activation, oxidation-reduction processes stimulation and the bio-membranes stability support.

2.5. Mechanisms of Actions of Macro-, Micro- and Ultra-Micro Elements which are included in «Grinization» complexes

The «Grinization» complexes include all vitally needed macro-, micro- and ultra-micro-elements which are compulsory components of the animal and vegetable matter being their source used in «Grinization» complexes production. With the scope of the «Grinization» complexes functional activity increase, some essential macro- and micro-elements are added, including: calcium, magnesium, zinc, manganese, silicium, vanadium, which are included into the complexes in the most solvable and acceptable form of chelates.

The deficit of calcium, manganese and silicium is the main cause of the osteoporosis. In the modern technology production, the named minerals are presented in very small quantities. The population as a whole, including children, uses the milky products in a negligible quantities and some part of the population due to the gastric diseases does accept milk at all. Excessive use of sweets, sugar containing drinks and coffee causes the greater export of the minerals with urine while in the conditions of high carbohydrates' consumption, the needs of the organism in the essential minerals are really high.

Manganese performs the significant anti-oxidative function. It is included in the structure of Mn-dependent super-oxide-dismutase which is the only anti-oxidant being based directly in the mythochondria where the intensive processes of oxidation and ATP synthesis occur. The Mn-dependent super-oxide-dismutase protects the mythochodria from the oxidant stress.

Magnesium takes an active part in the functioning of about 300 enzymes, which realize: the oxidation of fat acids, glucose metabolism, ATP synthesis. This element activates amino acids and, in such a way, takes part in proteins' construction (growth factor). Magnesium makes the muscles harder due to its possibility to activate cholinesterase, minimizes the emerging spasms and is the cofactor of the vitamins from the group B. Magnesium takes part in the formation of the catalytic centers in human brain, stabilizes the regulatory sites, in synthesis of neuro-specific proteins, in degradation of all neuromediators, — such as noradrenaline, acetylcholine and in synthesis of all neuropeptides in central brain. While there is the magnesium deficit, the memory and attention become weaker. Magnesium protects the central nervous system from the negative effect of the free radicals, toxic substances and various medicines. It diminishes the neurotoxicity and accumulation in the nervous tissues of the following microelements: berillium, nickel, lead and aluminium. Magnesium is the natural insulator on the way of the nervous pulse propagation, it control the activity of the voltage-dependent ionic chanel for calcium, potassium and sodium, prevents the emerging of the cardial arythmicity, optimizes the osmomolar processes in nervous and other tissues and extinguishes the swelling. Magnesium minimizes the risk of the momentary death.

Zinc is called the main mineral of the immune system. The deficit of this element causes the tymus' involution, the decrease of the tymocytes' number and suppression of their function, the decrease of the tymaline level in blood serum (zinc is needed for its activation); the decrease of the perypheric T-lymphocytes number, the diminishing of T-lymphocytes proliferation under the influence phyto-gemagmo-tynine, the decrease of their cytotoxic activity, the minimizing of T-heplers lymphocytes, the activity of EK-cells; the macrophages' function (phagocytosis and medium-cell killing), decrease of neutrophylic granulocytes (phagocytosis, chemotaxis) as well as the antibodies' production. Zinc is being included to the enzyme of alcohol-dehydrogenase and

insulin, takes part in the in the neuromediators' exchange and in the activity of some receptors in the central nervous system, provides the normal embryos' development and causes the bettering influence on the skin. Under zinc deficit, the risk of men's sterility and apoptosis of the weak cells increase significantly.

Selenium is the significant component of the enzymeative system of the glutation for the anti-oxidant protection and is included into the composition of 200 enzymes engaged in different bio-chemical reactions, demonstrates the immune-tropic, anti-teratogenic and anti-cancer properties, betters the functional state of muscles, especially the myocarditis, takes part in the tyreoidus gland's hormones' synthesis. Selenium's deficit in soil is the cause of the hearth deficiency in endemic zones.

Iodine takes part in the thyroidal gland's hormones' synthesis. The endemic goiter with the hypothyreosis occurs under the iodine deficiency as well as the arterial hypertensitivity. The iodine's deficiency in pregnants could cause the embryo's growth hindrance, the mental retardation and deaf-muteness in the neonatals, causes the spastic paralysis and initiates the auto-immune thyreoiditis as well as sterility.

Vanadium effect on the glucose exchange is the most studied phenomenon. It supports the glucose transport into cells and its action doesn't depend on the insulin presence what is especially significant for the insuline-non-dependent diabetics. The significant effect of vanadium is its ability to block the synthesis of the main enzyme of the cholesterine synthesis — the hydro-oximethyl-glutaril-CoA-reductase, e.g. vanadium, as well as statines, depresses the cholesterines; synthesis and causes its concentration in blood. Vanadium causes, as well, the decrease of the arterial pressure.

2.6. Mechanisms of Actions of other components which are included in «Grinization» complexes

The positive influence on the organism is being produced by the bi-phlavanoids, phytosterines and phytostanols which are included into the «Grinization» complex's content. The biologically active substances demonstrate the anti-inflammatory, spasmolythic, anti-microbial, anti-oxidant, immune-modulating and other properties. The biologically active substances of the «Grinization» phyto-mediators enhance the other processes and, under conditions of the stable use, form strong positive effect. The main mechanism of the phenol vegetable substances action, which influences the biological effect, is their ability to participate in the reversible oxidation-reduction reactions in the organism.

The Anti-Oxidant of the Phenol Compounds includes the following Mechanisms:

- Anti-free-radical (the interception of free radicals);
- Anti-lipo-peroxide (the interception of the peroxide oxidation radicals);
- Anti-oxygen;
- Peroxynitrite desactivation;
- Xantyne-oxidase and other radical-producing enzymes' activity;
- Metals' chelatazing.

The bi-phlavanoids in vegetables and fruits are situated regularly aside with the ascorbine acid. The bi-phlavanoids cause the ascorbine acid's storage in leucocites, in adrenal glands' core and in other organs, and cause more effective expenses under the ascorbine acid's deficiency in the organism. The phenolic compounds, being intruded into the organism, activize the detoxication processes in liver. The phlavanoids exceed, as to their anti-oxidant effect, the tocopheroles and carotinoids.

Phytosterins and phytostanols of the vegetable origin are called the analogs of the animal cholesterine. They are not consumed in the intestinal tract, but demonstrate trhe antagonism to the cholesterine taken from the food as well as to the endogenic cholesterine exported with the bile, diminishing its absorbability and causes the cholesterine extraction from the intestine. Due to the mentioned properties, the phytosterines and phytostanols produce the hypocholesterinemic action. The original technology of the ecological pure source of the sea and terrestrial origin, guarantees the supply of the dominating part of all nutrients in the biologically accessible forms of the colloids, water-solvable substances and micro-capsules. Due to the high level of consuming accessibility of all the nutrients, the conditions for the methabolic disorders' correction as well as for the immune-protecting and anti-oxidant actions as compared to the food materials obtained through the use of the traditional technology use. This is certified by the results of the multi-nutrient functional-peptide «Grinization» complex's studies at the facilities of the leading research institutes and clinics of Ukraine.

3. The Use of Multi-Nutrient Functional-Peptide «Grinization» Complex in the Medicinal Feeding and Complex Therapy of the Patients with Viral Hepatitis

Diet therapy is the permanently acting and secure component of the complex treatment of the liver diseases, including the viral ones[3, 4]. Adjusting the chemical proportions of the ration, which in turn, depends on the patient's needs, peculiarities of the disease and adding the products which possess high biological and medicinal activities, it becomes possible to stabilize the process as well as to

provide the emergence of the liver steatosis, steato-hepatitis, the toxic viral hepatitis and the starting phases of the cyrrosis in the great majority cases. As the multi-nutrients functional-peptide «Grinization» complex contains the different biologically active substances being able to influence the stage of the liver and that of the organism as a whole, and this makes to use the complex during the treatment of the hepato-biliar system at the different stages of the disease.

3.1. Influence of «Grinization» complex on the different liver's functions in patients with viral hepatitis

Main liver function is to deactivate toxic products in a body. Therefore, condition of its detoxicating function is one of the most significant indicators of hepatoprotective ability of liver cells. Since shortening of thiopental sleep duration indicates increase of liver detoxicating function, we have studied impact of «Grinization» complex activity on this indicator by changing thiopental sleep pattern. Thiopental sleep was induced in rats by intravenous administration of natrium thiopental in the dosage of 20 mg/kg. Duration of side positioning of the animals was determined after natrium thiopental has been administered, as well as of intact rats, and after administration of «Grinization» complex for two weeks. Daily doses constituted 200 (100 mg of «Grin Mix» + 100 mg of «Grin Pro») mg/kg, 400 (200 mg of «Grin Mix» + 200 mg of «Grin Pro») mg/kg and 600 (300 mg of «Grin Mix» + 300 mg of «Grin Pro») mg/kg.

Results of the study on impact of the Complex on thiopental sleep duration in rats are presented in Table 4.

Table 4. Duration of Thiopental Sleep in Rats after Administration of «Grinization» Complex

Preparation	Dose mg/kg	Number of Animals	Falling Asleep (after how many minutes)	Duration of Sleep (minutes)
Natrium Thiopental	20 mg/kg	10	1 min	37
Natrium Thiopental accompanied administration of Grinization Complex in 200 mg/kg dose	20 mg/kg	10	3 min 30 sec	15
Natrium Thiopental accompanied by administration of Grinization Complex in 400 mg/kg dose	20 mg/kg	10	3 min 50 sec	10
Natrium Thiopental accompanied by administration of Grinization Complex in 600 mg/kg dose	20 mg/kg	10	did not fall asleep	did not sleep at all

Results indicated in the Table above prove that complex «Grinization» is highly effective in increasing detoxicating function of the liver.

During the chronic viral hepatitis, the «Grinization» complex is used in the cases of combined chronic hepatitis and the liver steatosis. Steatohepatitis and at the performance of the regular anti-viral therapy of the chronic C hepatitis with the use of the preparations of the recombinant interferon combined with Ribavirin and other anti-viral means. The use of the «Grinization» complexes under the viral hepatitis allows to create the comfortable conditions for the immune system whose state determines the way of disease progress as well as its results. During the viruses' interaction with the macro-organism, the different immune reactions take place which are being oriented, from one side, to the elimination of the disease initiator, and, from the other side, the viruses cause the inflammation processes in the organism, the hindrance of the apoptosis' reactions, what cause in turn the chronic forms of the disease. According the clinical studies' results, under the use of the «Grinization» complexes at the chronic viral hepatitis, the apoptosis reactions are being activated as a result of the emerging of the CD-95 receptors. This process is accompanied by the improvement of the liver functional state which is demonstrated clinically through the normalization of the liver state and diminishing of the liver enzymes' system activity as well. The deficiency of only one non-restituable nutrient component could cause the decrease of the immune defense system.

The presence of the certain number of amino acids is significant for the restitution of the immune system's cells, interferon synthesis process and other factors' realization of immune defense system. The decrease of the full form protein consumption is one of the causes of the secondary immune-deficiency state emerging. The significance of the ascorbine acid presence for the immune system is supported by the fact that its concentration in the neutrofile granulocytes is by 150 times higher than in the blood serum. The significance of the retinole's and carotinoids' role is supported for the cases of cells' differentiation, the DNA synthesis increase, proliferation decrease which all serve to the stability of the organism under the infection attack.

The carotinoids are the protectors of the immune-competent cells' segregation, normalization of the immune-globulines' synthesis, including the secretory immune-globuline A, immune protection of some factors of specific and non-specific protection and lysomes' activation in phagocytes. The supply of the **folic acid** is significant for the regular functioning of the organism's immune competent organs. The decreased use of the folates causes the lowering of the DNA methylation level in lymphocytes. The additional use of the folic acid assists the reparation of the destructed DNA elements. The expressed ability to the immune-tropic action is stated for the **selenium** and **zinc.** The **selenium** deficiency is responsible for the viral infection intrusion into the organism, including the new ones modification. **The zinc** deficiency causes the thymus involution, the tymocytes' number decrease, their functioning inhibition and the tymulin's level in the blood serum decrease. **Zinc** is the immune modulator, protector of the auto-immune reactions' formation,

what is significant for the patients with the viral hepatitis who undergo the antiviral therapy. The interferon ordering initiates the risk of the auto-immune reactions and that of the overlap syndrome (what signifies the combination of the viral hepatitis and the auto-immune one). The nutrients which use the immune cells, take them from blood and their level is supported by the substances taken from the outer space.

As the chronic viral hepatitis is the disease which is accompanied by the prolonged viruses' persisting, the adequate state of the organism immune system support requires the stable input of the nutrients during all the life remained. No one synthesized immune stimulator couldn't feed the immune cells and give the material for the other immune factors creation. The «Grinization» includes all the non-restituable amino acids, fat acids, vitamins and minerals for the reparation of the immune system's cells and the anti-oxidants for the protection from the free radicals. In addition, the «Grinization» complex contains as well the transfer factors which influence initially the adequacy of the immune system. These transfer factors are being contained in eggs in sufficient quantity but they are destroyed during the thermal processing.

It was shown experimentally that «Grinization» protects the emerging of some complications under the use of antiviral therapy, diminishes the demonstration of the extracellular destruction under the viral hepatitis. The thrombocytopenia is one of the extracellular demonstration of the chronic viral hepatitis C which hinders the use of the antiviral therapy or even demands the stop of it[5]. The causes of the thrombocytopenia's emerging under the viral hepatitis C are not understood well. It was stated that hepatitis C virus RNA is detected often in the thrombocytes taken from patients with viral hepatitis C, while the blood serum taken from 66 % of all the hepatitis C patients contains the antibodies to the thrombocytes. The thrombocytes in human organism fulfill the following main functions: participation in the haemostasis processes, blood coagulation, local inflammation reaction and immunity regulation, the vasoconstrictors' extraction. The main regulator of the thrombocytopoesis is the thrombopoetin — the polypeptide which are represented in great number in the liver which is the place for its synthesis. The causes of the thrombocytopenia emerging in the patients with viral hepatitis C are the following: hypersplenism, the decrease of thrombopoetines' synthesis, immune-mediated thrombocytes' clearance, the influence of the viruses on the cells-predecessors of the thrombocytes — megacaryocytes due to their replication in these cells.

The medicinal thrombocytopenia could emerge under the use of different medicines, including interferon. The main mechanism of the medicinal thrombocytopenia pathogenesis is assisted by the thrombocytes' destruction by the complement which is activated during the preparation's reaction with the antibodies to it. After the preparation use's stop, the thrombocytes'

content in blood is being normalized. The antibodies creation to the thrombocytes in the patients with viral hepatitis C could occur due to destruction of the membrane glycoproteins by the viruses.

It was stated that the use of the «Grinization» complex diminishes the probability of the thrombocytopenia in the patients with viral hepatitis C as well as the level of its presentation and the frequency of its emergency during the application of the antiviral therapy what enhances the efficacy of the latter.

3.2. The algorithm of the «Grinization Mix» and the «Grinization Pro» use for the patients with chronic viral hepatitis C during the antiviral therapy realization

There are 4 Variants of the «Grinization Mix» and the «Grinization Pro» use for the patients with chronic viral hepatitis C during the antiviral therapy realization.

Variant 1. The patients with the absence of the thrombocytes number‘s changes are recommended the prophylactic doses of Grinization (Grin Mix — from 5 to 10 ml per day, Grin Pro — from 3 to 7 capsules per day or the 2.5 — 5 mg of the powder per day). While the number of thrombocytes is diminished slightly, the dose of Grinization is being incgeased.

Variant 2. The patients with small thrombocytes’ number in blood decrease (not less than 100×10^9 in 1 liter) are recommended during the antiviral therapy application the medium doses of Grinization (Grin Mix — 15 ml per day, Grin Pro — 10 capsules per day, or 7 g of the powder per day). With the tendency to the thrombocytes number’s decrease, the dose should be diminished to the level of the prophylactic one.

Variant 3. The patients with the serious decrease of the thrombocytes number’s in blood (less than 90×10^9 in 1liter) before the antiviral therapy application during 14 days are recommended to use the high doses of Grinization (Grin Mix — from 25 to 33 ml, Grin Pro — from 12 to 16 g per day), till the number of thrombocytes is increased up to 100×10^9 in liter and more. For all the period of the antiviral therapy application the Grinization is recommended in the medium doses. While the thrombocytes number is stabilized, the Grinization doses should be diminished up to the prophylactic ones.

Variant 4. The patients with the thrombocytes number’s decrease up to 80×10^9 in liter and less, without the antiviral therapy stop, the Grinization is recommended in high doses and with the thrombocytes number increase, the treatment is still performed at the background of high Grinization doses. While the number of thrombocytes is being stabilized in the range of (100–150 $\times 10^9$ in liter), the Grinization dose is diminished to the medium level and with the tendency to the thrombocytes number decrease the dose should be increased.

The positive influence of the Grinization on the thrombocytes number is caused by the immune-modulating, cytoprotecting, antioxidant and the apoptosis-modulating action of the biologically active components of the Grinization multi-nutrient complex.

Presented materials acknowledge doubtless advisability to use «Grinization» in complex therapy of viral hepatitis, as well as in prevention and treatment of complications thereof.

4. Anti-Influenza Action of Multi-Nutrient Functional-Peptide Complex «Grinization»

Influenza is the most widespread acute infectious disease of humans. Annually influenza is on account for morbidity rate and mortality increase all over the world. For example, every winter about 300000 patients in the USA are hospitalized and 30000–40000 patients die as a result of influenza infection. The morbidity and mortality rates due to influenza infection are increased dramatically in case of pandemic. In the 20th century there were 3 pandemics — the pandemic of Spanish influenza in 1918, caused by a virus of antigenic formula H1N1; the pandemic of Asian influenza of 1957, caused by a virus of antigenic formula H2N2; the pandemic of 1968 year caused by a virus with antigenic formula H3N2. These pandemics are characterized by a morbidity rate from 30 % to 60 % of the population and accompanied by dramatic increase of pneumonias number and general mortality. The pandemic of Spanish influenza of 1918, which took lives of 1 % to 2 % of all population of the Earth, was especially serious. Nowadays almost the whole world is stricken with the first influenza pandemic of the third millennium caused by strain A/California/04/2009 (H1N1).

Prior to the beginning of this pandemic, annually during the first quarter of the year up to 10 million people in Ukraine, 52 % of which were children, applied for a medical help concerning seasonal influenza and ARVI. It is necessary to emphasize that influenza is quite dangerous disease for children due to the high probability of complications development. Besides, modern anti-influenza agents have series of restrictions for paediatric application. Therefore, research and development of effective anti-influenza agents, and also elaboration of methods reducing influenza morbidity rate, seriousness and risk of disease run — are enormously actual problems of medical science and public health services.

Medications enhancing immune response, increasing non-specific organism resistance and stabilizing bio-membranes play an important role in the fight with influenza and other acute respiratory virus infections. Multi-nutrient functional peptide complex «Grinization» has such characteristics. It advantageously differs from most of existent special products (as it contains natural extractions and extracts of animal and vegetable origin, which have all necessary matters in their natural interactions and proportions).

Therefore we had studied anti-influenza action of «Grinization» complex.

4.1. Protective Action of «Grinization» Complex during Dangerous Form of Experimental Influenza

The effect of «Grinization» on protection of lives of animals from the destructive influence of influenza infection, modelled by means of infection of experimental mice with the highly virulent influenza virus strain A/PR/8/34 (H1N1) was studied as follows. Mice of the experimental group received orally 150 mg/kg of «Grin Mix» and 2 hours later they received 150 mg/kg of «Grin Pro» daily during 7 days before infection with influenza virus A/PR/8/34(H1N1) and following 14 days. Mice of the control group received placebo. Four animals in each group (experimental and control), were infected intra-nasally under the light ether anaesthesia with tenfold dilutions of virus containing allantoic liquids from $1x10^{-1}$ to $1x10^{-7}$ in the volume of 0.05 ml. Death of animals was registered within 14 days after infection. LD_{50} was calculated by the modified Kerber's method by the formula:

$$-\log_{10} LD_{50} = -L - d(S-0.5),$$

where L — initial dilution of an infecting dose; d — difference between consecutive dilutions in $\log_{10}$; S — sum of proportions of test-objects, which have yielded positive result (i.e. quantity of the died animals in relation to infected with the same dose).

The effect of «Grinization» on dynamics of mice death is presented in the fig. 1 as cumulative $\log_{10}$ LD_{50}.

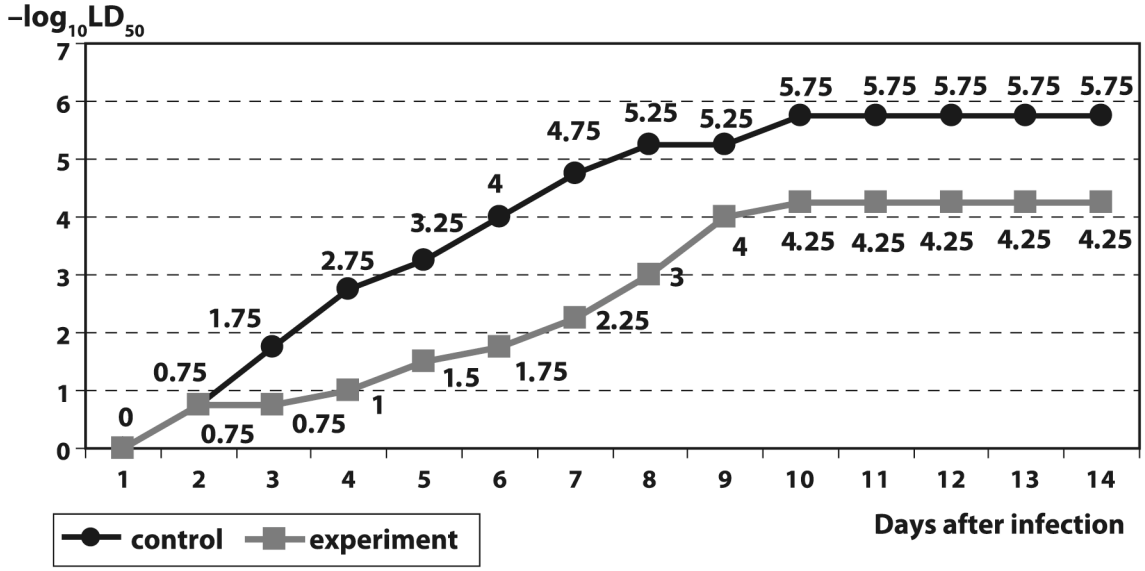


Fig. 1. Protective effect of «Grinization» on lethal form of experimental influenza in mice infected by strain A/PR/8/34 (H1N1).

Date analysis shows that animals in both groups started to die on the 2nd day after infection, and, since the 3rd day and till the end of examination, the expressed protective effect of

«Grinization» was observed. Thus, on the 6-8th days when the death due to a virus infection was no longer observed, differences of cumulative $\log_{10}$ LD_{50} between the control and experimental groups were 2.25–2.5 $\log_{10}$ LD_{50}, i.e. to cause the death of 50 % of animals by this term in the group of animals receiving «Grinization», the infecting dose should be 160-320 times higher than a dose necessary for the achievement of the same effect in the control group.

At the end of observation the difference was 1.5 $\log_{10}$ LD_{50}, i.e. for the achievement of identical effect (death of 50 % of animals) in the group of animals receiving «Grinization» prior and after the infection, the quantity of necessary virus was 32 times higher, than in the control group. If we compare the level of lethality in these groups, then the group of animals receiving «Grinization» complex had 21.4 % less of died mice, than the control. Extrapolating these results to people, it is possible to expect that those, who accept «Grinization» complex during the influenza epidemic period are protected against serious forms of influenza to higher extent. It is especially important for people from risk groups: medical workers, teachers, trade and transport workers, persons older than 65 years.

Thus, it was observed the fact that serious forms of the experimental influenza infection are tolerated better and the mortality due to influenza is lower in the group of animals, which were infected and the development of infectious process occurred during reception of «Grinization» complex.

4.2. Influence of «Grinization» Complex Use on Viral and Bacterial Titres in Lungs during Experimental Influenza

For revealing of possible mechanisms of protective anti-influenza effect of «Grinization» it was necessary to study the effect of its use on infectious virus quantity and lungs bacterization, and also on morphologic changes in lungs and some other organs at modelling of the nonlethal form of influenza infection by means of mice infection with the highly virulent influenza virus strain A/PR/8/34 (H1N1).

The studies were conducted as follows. Mice of the experimental group received orally 150 mg/kg of «Grin Mix» and 2 hours later they received 150 mg/kg of «Grin Pro» daily during 7 days before infection with influenza virus A/PR/8/34(H1N1) and following 14 days. Mice of the control group received placebo. Mice of the experimental and control groups were infected intra-nasally under the light ether anaesthesia with allantoic liquid containing virus A/PR/8/34 (H1N1) in a dose of 0.5 LD_{50}.

In 1, 3, 5, 7, 10 and 14 days after infection (a.i) mice from both experimental and control groups were taken for experiments: 4 mice for virological, 3 — for morphological and 3 — for

bacteriological experiments using draining of blood under ether anaesthesia. Requirements of the European convention on protection of the vertebrate animals used for research and other scientific purposes (Strasbourg, March 18th, 1986) were satisfied while carrying out the experiments.

The amount of infectious virus in lungs was defined by titration of 10 % homogenates on tissue culture of chorio-allantoic membranes (CAM) of 11–13-days old chicken embryos. TID_{50} was calculated by the modified Kerber's method by the formula mentioned above.

The effect of «Grinization» on virus accumulation in lungs of infectious virus accumulation in lungs of the infected mice are presented in fig. 2.

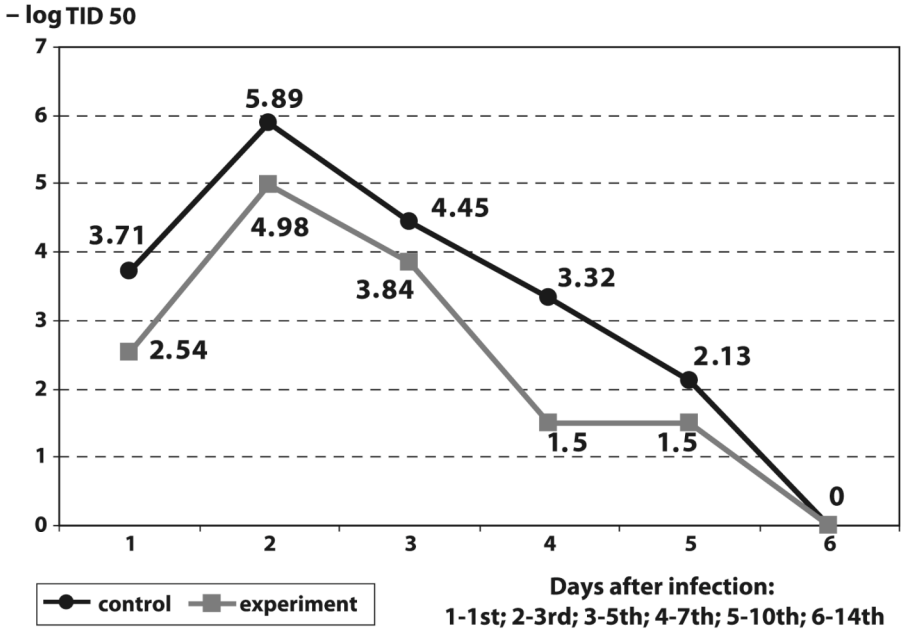


Fig 2. The effect of «Grinization» application on dynamics of infectious virus accumulation in lungs of the infected mice.

These results show that maximum level of virus in lungs of animals of the control group was accumulated on the third day, then it was gradually decreased and on the 14th day the virus was not already detected, as well as in the experimental group. Animals receiving «Grinization» had much lower level of infectious virus in lungs since the 1st day of infection and till the 10th day after infection, than control animals. Differences accounted for 0.9 — 1.15 $\log_{10}$ TID_{50} (i.e. 8–14 times) on the 1st, 3rd and 7th days and nearby 0.6 $\log_{10}$ TID_{50} (i.e. approximately 4 times) on the 5th and 10th days.

For bacteriological analyses next commercially available culture media were used: Endo agar, elective saline agar, blood agar, thioglycolic medium and beef extract broth (BEB) with 1 % of glucose. Chocolate agar was used for isolation of haemophilic flora.

Lungs were selected from 3 individuals, they were weighed, and 10 % homogenates were prepared on sterile 0.85 % NaCl solution and analysed individually. Extracted microorganisms were identified in accordance with Bergey classification.

The growth of individual colonies of *S.aureus* was observed in some samples on the 10th day after infection with influenza virus. On the 14st day the average concentration of *S.aureus* in experimental samples was less than 10^3 CFU/g (CFU – colony forming unit) while in control samples the concentration of bacterial contamination with association of *S.epidermidis* and *S.aureus* was higher - 10^4 CFU/g.

Thus, application of «Grinization» has led to the decrease of bacterial contamination of lungs of animals in case of developed bacterial infection. Besides, only pathogenic staphylococcus was detected in this group, while bacterial contamination with association of both pathogenic and opportunistic pathogen microorganisms was detected in the control.

Obtained results show that influenza virus is reproduced worse in lungs of animals receiving «Grinization», than in lungs of mice, which have not received it. It also applies to potential causative agents of bacterial complications. Thus, application of «Grinization» complex increases protection of an organism not only against influenza, but also against possible bacterial complications.

4.3. Influence of «Grinization» Complex Use on Morphological Damages of Internal Organs of mice during Experimental Influenza

For morphological analyses internal organs of mice were fixed in 10,0 % neutral formalin, they were processed by the standard histological technique, paraffinic sections were stained in haematoxylin — eosin. Changes in internal organs of mice were assessed by semi-quantitative method with division into four gradations (points) — not detected (0), 1 +, 2 +, 3 +.

Statistical analysis of the obtained research data was performed by the methods of parametric and nonparametric statistics using software «EXCEL» (Microsoft, 2003, USA) and «STATISTICA 6.1» (StatSoft Inc., 1984-2004, USA).

The animals were divided into such groups: group I (control) included infected animals which got water (placebo) through the probe twice a day every day; group II consisted of infected animals which were administered multi-nutrient functional and peptide complex (MFPC) «Grinization». Suspensions of «Grin Pro» (150 mg per 1 kg of weight) and «Grin Mix» (150 mg per 1 kg of weight) in the volume of 0.1 ml were administered through the probe every day. The course lasted for 7 days before infecting and for 14 days after it.

The lungs of animals as target organs were examined and verification of the flu model was performed on the 1, 3, 5, 7, 10, 14 days after infecting by virological, bacteriological (see above) and morphological methods. Other organs (the liver, kidneys, spleen, and heart) were investigated on the same time periods by morphological methods and the liver was also examined virologically on the 7th day and at the end of the experiment.

Presence of interstitial histiomacrophage infiltration of interalveolar walls, pneumonia, acute bronchitis, congestion of vessels, hemorrhages, peribronchial and perivascular lymphoid infiltration, foci of emphysema and dystelectasis was considered in the assessment of morphological changes in the lungs.

Morphological differences between the animals from group I and group II were significant. Fig.3 shows dynamics of inflammatory process development in both groups. In group I inflammatory changes in the lungs made rapid progress and reached a maximum on the 10th day. Intensity of inflammatory changes in the lungs of animals which were administered MFPC «Grinization» was much lower and the alterations varied throughout the experiment; at the end of it the lungs were fully regenerated.

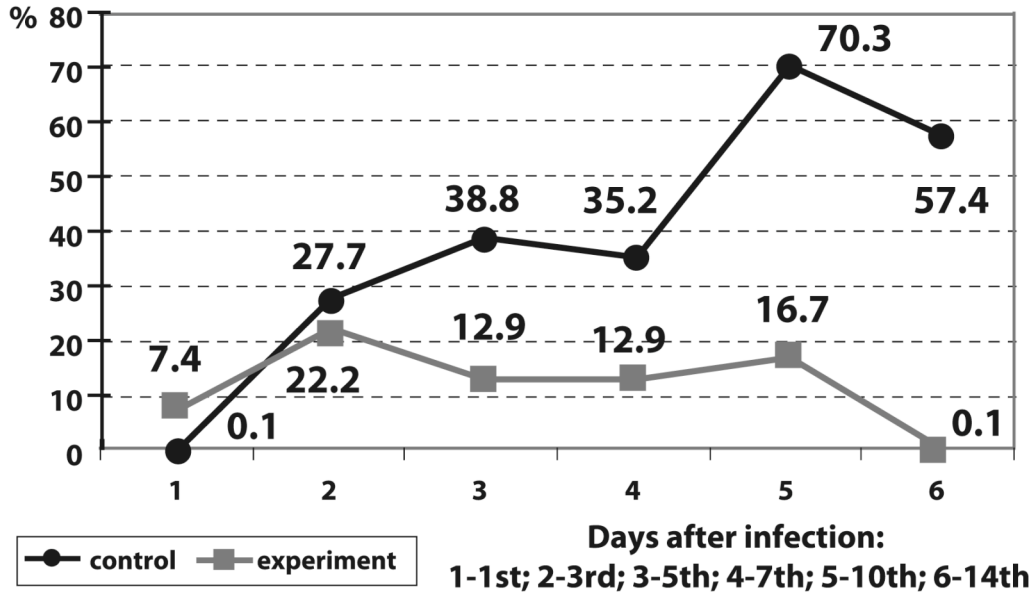
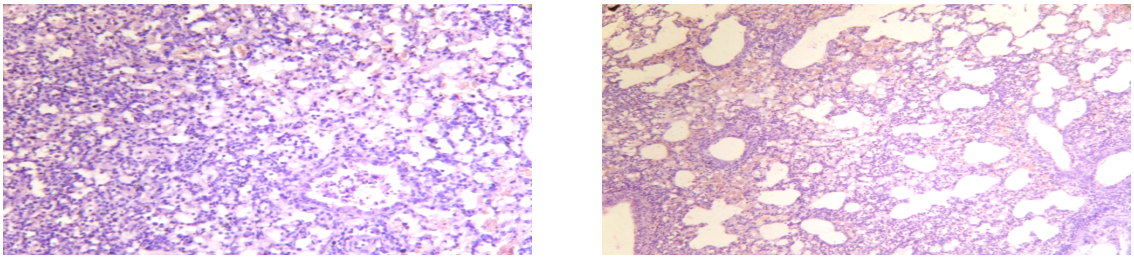


Fig. 3. Dynamics of inflammatory process development in the lungs of animals from a group of control and animals administered MFPC «Grinization».

In 24 hours after infecting lesions in the lungs of all animals were — insignificant — minor hemorrhages, slight peribronchial cuffing and perivascular lymphoid infiltration. Mucous membrane of the bronchi was intact. The major differences of animals from group II were presented by more intensive peribronchial and perivascular lymphoid infiltration, focal interstitial histiomacrophage infiltration of interalveolar walls, which prevailed in the lesion picture.

On the third day both groups manifested progression of the pathological process and on the whole differences in affection of the lungs between groups were minor in terms of volume. However, qualitative characteristics differed significantly. In group I there were found marked dystrophy and desquamation of the bronchial epithelium; some cell debris congregated in the bronchial lumens;

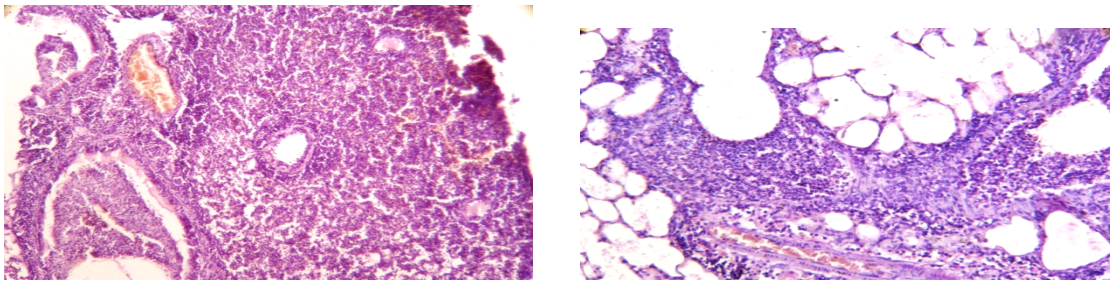
purulent bronchitis and bronchopneumonia were observed to develop. In group II interstitial infiltration of interalveolar walls, peribronchial and perivascular lymphoid infiltration were increased, the picture was complemented with singular pneumonia foci (Fig. 4, a,b).



a) b)

Fig. 4. a) Acute bronchitis and bronchopneumonia, marked inflammatory infiltration with mild hemorrhagic component in the lungs of animals infected with the flu, the third day. Hematoxilin-eosin staining, magnification × 200; b) focal and diffuse interstitial infiltration with mild hemorrhagic component in the lungs of infected animals administered MFPC «Grinization», the third day. Hematoxilin-eosin staining, magnification × 100.

From the fifth day of the experiment qualitative and quantitative differences between groups were growing and becoming reliable. In animals from group I inflammatory changes kept on progressing — purulent bronchitis manifestations intensified, number of bronchopneumonia foci and their sizes increased, hemorrhagic pneumonia foci with no tendency to delimitation appeared. Pericanalicular lymphoid infiltration and interstitial infiltration of interalveolar walls were inconstant and insignificant. In group II focal and diffuse mild interstitial infiltration of interalveolar walls prevailed in the morphological picture of the lung lesion just as it was observed in the previous terms. The intensity of peribronchial and perivascular lymphoid infiltration was slightly decreased, bronchopneumonia foci remained small and inconsiderable in numbers and clearly localized (Fig.5, a,b).

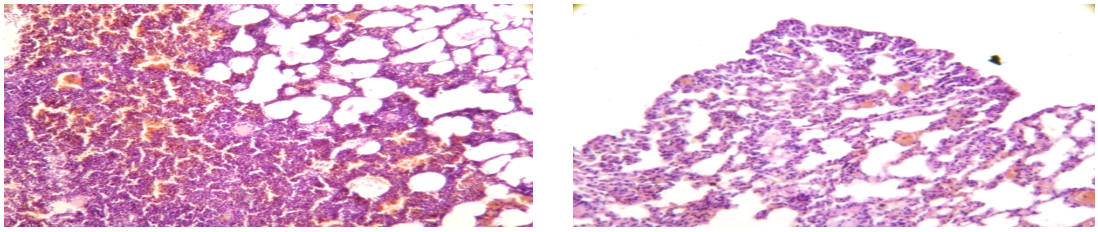


a) b)

Fig.5. a) Acute bronchitis and bronchopneumonia focus in the lungs of the animal infected with the flu, the fifth day. Hematoxilin-eosin staining, magnification × 100; b) acute bronchitis with a minimal peribronchial inflammatory response in the lungs of infected animals administered MFPC Grinization, the fifth day. Hematoxilin-eosin staining, magnification × 200.

On the seventh day inflammatory process progressing in group I was accompanied with hemorrhagic component intensifying in combination with marked purulent bronchitis and confluent

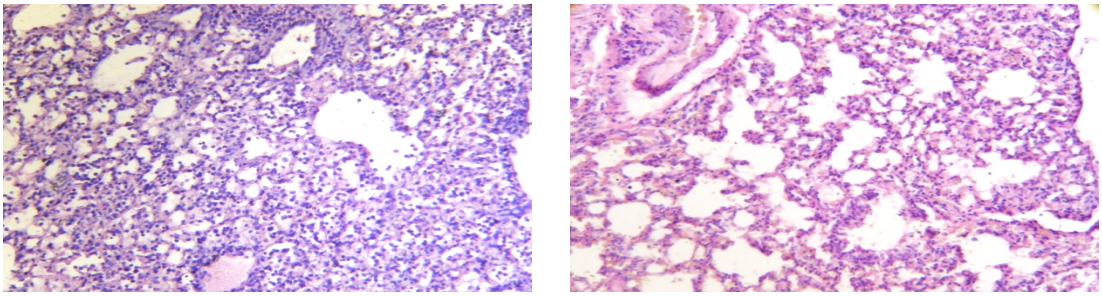
bronchopneumonia On the contrary, group II manifested emphatic evidence of inflammatory changes regression; changes in the form of moderate focal and diffuse infiltration of interalveolar walls were dominating; pneumonia and bronchitis foci were small and single; vascular responses were minimal; observed were minor dystelectasis (Fig.6, a, b).



a) b)

Fig. 6. a) Focal hemorrhagic pneumonia in the lungs of animas infected with the flu, the seventh day. Hematoxilin-eosin staining, magnification × 100; b) interstitial infiltration, vascular distention and congestion in the lungs of infected animals administered MFPC «Grinization», the seventh day. Hematoxilin-eosin staining, magnification × 200.

The tenth day was characterized by maximum intensity of pathological changes in the lungs of animals from group I — massive confluent foci of hemorrhagic pneumonia and bronchopneumonia. Two animals from that group were found to have macrofocal changes that resembled the honeycomb lung with comb walls lined with cubical epithelium. The changes in the lungs of animals from group II administered MFPC «Grinization» were reduced to slight interstitial responses against the background of air parenchyma with focal slight vascular congestion, small dystelectasis foci and repeated intensified peribronchial and perivascular lymphoid infiltration (Fig. 7, a,b).



a) b)

Fig. 7. a) Bronchopneumonia focus in the lungs of animas infected with the flu, the tenth day. Hematoxilin-eosin staining, magnification × 200; b) focal interstitial infiltration in the lungs of infected animals administered MFPC «Grinization» the tenth day. Hematoxilin-eosin staining, magnification × 200.

On the fourteenth day in group I the changes were subtotal, purulent bronchitis and bronchopneumonia manifestations as well as hemorrhagic pneumonia remained unchanged. When exposed to MFPC «Grinization» the lungs of animals showed either full regeneration of lung

aeriality or minimal vascular and interstitial responses combined with marked peribronchial and perivascular lymphoid infiltration.

Fig.8 shows comparative analysis of the entire complex of inflammatory lung changes intensity in both groups at each observation term.

According to the diagram inflammatory changes in the lungs of animals of group I were worsening beginning with the third day of the experiment and on the fourteenth day they reached 77% of maximum possible level, whereas animals from group II, which were administered MFPC «Grinization», demonstrated decrease in their intensity and on the fourteenth day it amounted to only 22.9%, i.e. it was 3.3 times lower than in control.

Differences between groups appeared to be even more demonstrative in overall estimate of only 3 indices — intensity of interstitial infiltration of interalveolar walls, bronchitis and pneumonia (Fig.8). On the first day of the experiment maximum intensity of inflammatory responses in the lungs (it should be emphasized that it was interstitial infiltration) was high in group II, whereas in group I inflammatory responses were minimal. On the fourteenth day inflammatory changes reached a maximum in group I (bronchitis, hemorrhagic and bronchopneumonia), while in animals administered MFPC «Grinization» they were minimal.

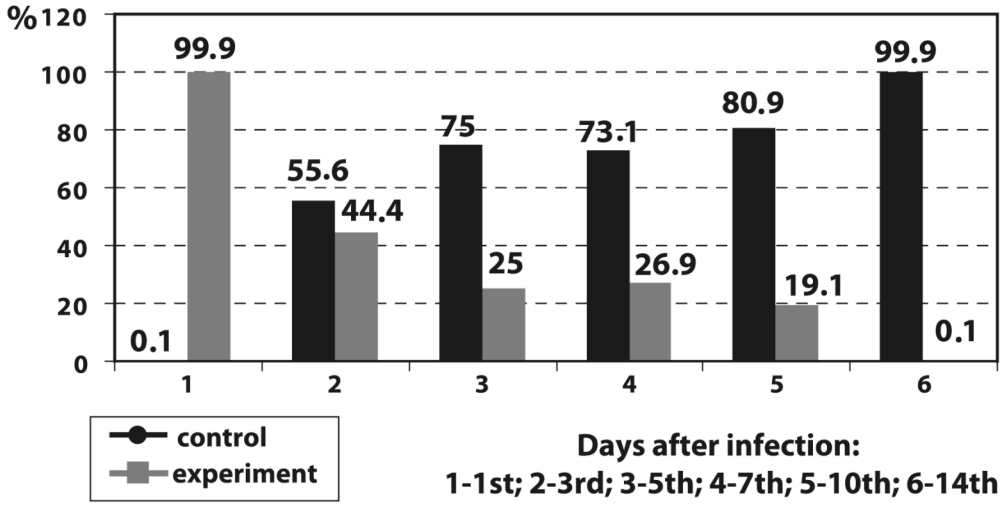
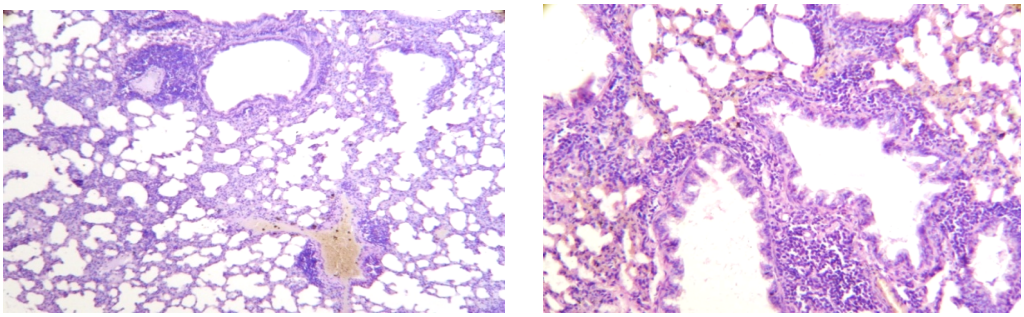


Fig.8. Comparative analysis of inflammatory changes intensity (interstitial responses, bronchitis, pneumonia) in the animal from a group of control and the group that got MFPC «Grinization».

Comparison of findings in the animals infected with A/PR/8/34(H1N1) influenza virus and animals administered MFPC «Grinization» against the background of influenza virus infecting revealed considerable differences between groups. Group I demonstrated marked disease progressing with typical inflammatory manifestations in the form of acute bronchitis, bronchopneumonia and hemorrhagic pneumonia. Changes associated with development of non-specific histiomacrophage responses and cell immunity manifestations in the form of perivascular and peribronchial lymphoid

infiltration were prevailing in group II. Against this background bronchitis and pneumonia progressing was minimal and their outcome came faster and earlier. In addition, maximum virus loading in the lungs was aligned with maximum lymphoid infiltration and decrease in virus concentration was accompanied with decreased activity of interstitial and lymphoid responses. Inflammatory changes intensity in the lungs of animals from group I were increasing in spite of decrease in virus concentrating.

Such a component of inflammatory response to influenza virus infecting as peribronchial and perivascular lymphoid infiltration is noteworthy (Fig.9).



a) b)

Fig. 9. Perivascular *(a)* and peribronchial (b) lymphoid infiltration in the lungs of animals infected with influenza virus. Hematoxilin-eosin staining, magnification (a) × 100; (b) × 200.

Fig. 10 shows intensity of perivascular and peribronchial lymphoid infiltration which accompanies inflammatory process development in the lungs. The intensity of perivascular and peribronchial lymphoid infiltration varied throughout the experiment depending both on the date of observation and receiving the drug.

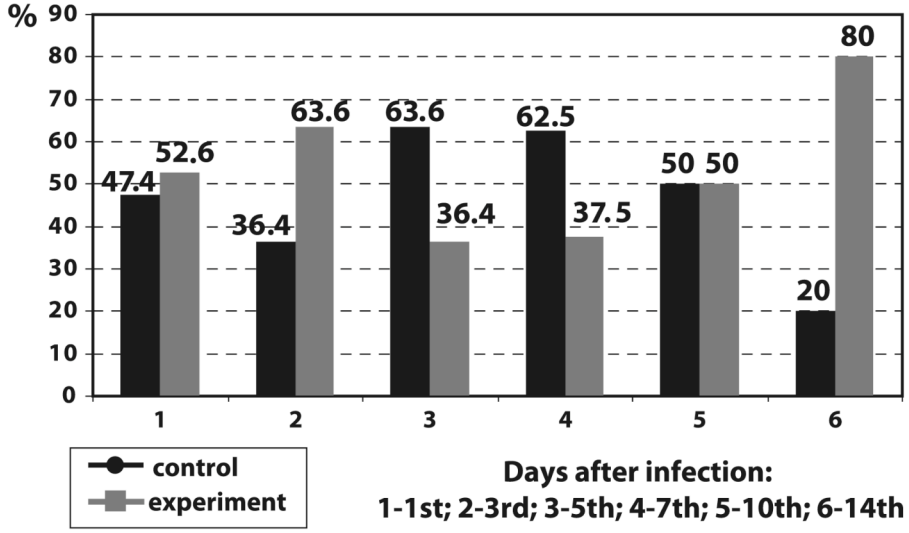
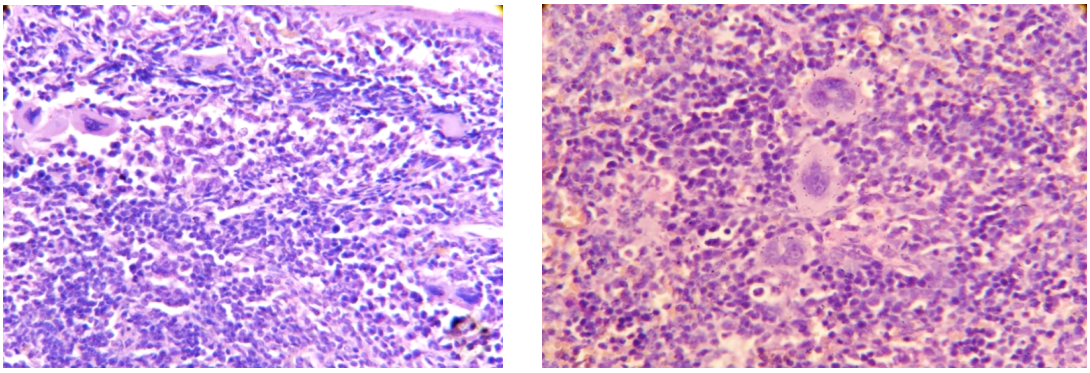


Fig. 10. Comparative analysis of perivascular and peribronchial lymphoid infiltration intensity in the lungs of animals from a group of control and the group that got MFPC «Grinization».

Fluctuations in lymphoid infiltration volume in animals from both groups are wavelike. In group I lymphoid response on the 1 and 3 days is lower than in group II, on the 5 and 7 days it increases and remains stable then decreases again by the end of the experiment. In animals from group II lymphoid infiltration rises by the third day then decreases on the 5 and 7 days and grows again by the end of the experiment. It is in accord with virological and bacterial findings — in group II activity of lymphoid responses is higher during the highest concentration of the virus in the lungs and highest activation of bacterial flora. Infected animals from the group of control show marked suppressive viral activity which results in immunodepression and activization of viral and bacterial infection.

It appears possible to judge whether increase in proliferative activity in the lymphoid tissue is systemic on the ground of assessment of megacaryocytes (change markers of proliferative activity in the lymphoid tissue of experimental animals) dynamics in the spleen.

In group I observed are megacaryocytes size reduction, basophily of the cytoplasm, reduced and hyperchromic nuclei which are the signs of suppressed proliferation. In group II the cell and nuclei are big with distinct nucleolus which testifies increased proliferative activity of the lymphoid tissue (Fig.11).



a) b)

Fig. 11. Megacaryocytes of the spleen a) animals infected with influenza virus; b) animals infected with influenza virus that were administered MFPC «Grinization». Hematoxilin-eosin staining, magnification × 400.

Fig. 12 shows dynamics of spleen megacaryocytes quantitative characteristics alterations. It is obvious that their dynamics is the same as in perivascular and peribronchial lymphoid infiltration study. Hence, perivascular and peribronchial lymphoid infiltration condition reflects systemic reaction of the lymphoid tissue of mice to maximum concentration of the virus in the lungs and bacterial flora activation in case of MFPC «Grinization» application which results in more adequate and concordant immune response in animals from group II.

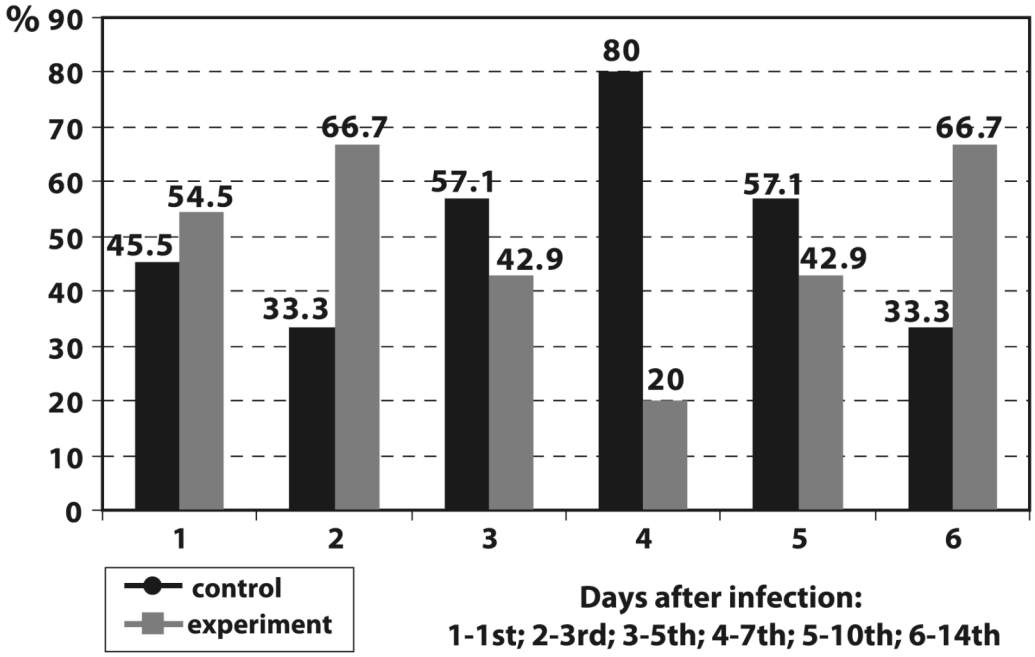
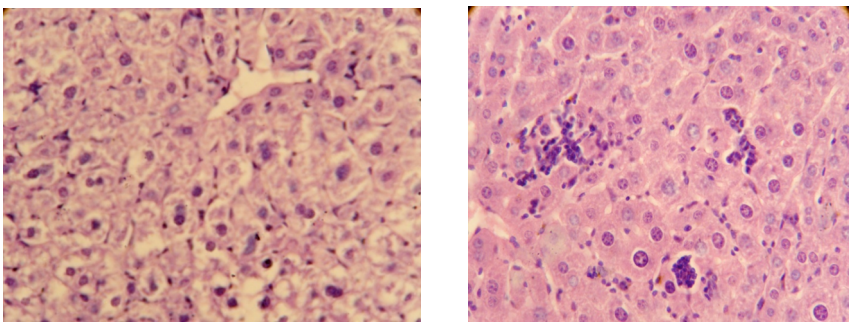


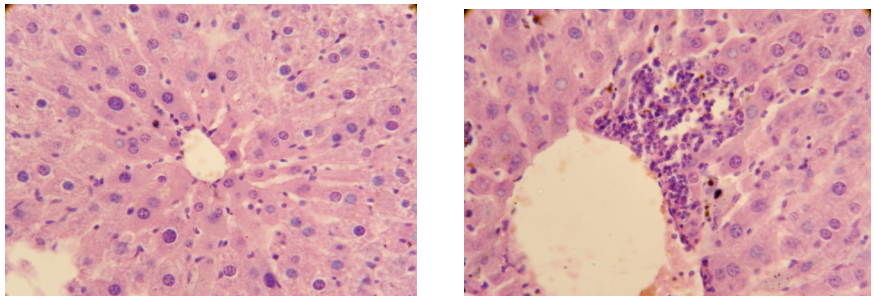
Fig. 12. Comparative analysis of spleen megacaryocytes proliferation rates in animals from the group of control and animals administered MFPC «Grinization».

This phenomenon can be explained having postulated that MFPC «Grinization» promotes higher resistance of the lymphoid tissue to infectious stress and appropriate active lymphoid tissue involvement in protection which allows major localizing infection as early as on the 1st and 3rd day. All in all, the curative effect of MFPC «Grinization» manifested itself by major inflammatory process reduction, adequate immune response which substantially improves influenza infection course in whole and reduces the risk of adverse outcome.

Presence of protein-hydropic degeneration (Fig.13, a), punctuate infiltration and necrosis, reaction of hepatic macrophages (Fig.13, b), nuclear polimorphysm (Fig.13, c), inflammatory infiltration of portal tracts and perivenulat infiltration (Fig. 13, d) were considered in assessment of morphological alterations in the liver of mice.



a) b)



c) d)

Fig.13. Liver of A/PR/8/34(H1N1) infected. a) intralobular protein- hydropic and ballon degeneration of the liver; b) small intralobular infiltrates and marked reaction of hepatic macrophages; c) polimorphysm of hepatocytes nuclei; d) mononuclear infiltration in the wall of the central vein. Hematoxilin-eosin staining, magnification × 400.

Microscopic investigation in mice from group I revealed presence of vessels congestion, small numerous intralobular and perivenular infiltrates, moderate focal protein- hydropic degeneration combined with polimorphysm of hepatocytes nuclei, inconstant lymphoid infiltration of portal tracts. Marked activation of histiomacrophage elements — hepatic macrophages must be emphasized. Homotypic moderate inflammatory alterations remained in the liver on the 3rd, 5th, 7th and 10th days. Presence of inflammatory mononuclear infiltration in walls of central veins, which is typical for a viral infection, goes in evidence of massive lesion of vascular endothelium. On the 14th day inflammatory changes intensity decreased, but protein- hydropic degeneration and reaction of hepatic macrophages remained and were even increased.

On the 1st day changes in the liver of animals from group II were insignificant compared with control. On the 3rd day they reached a maximum and were much higher compared with control, but on the 5th day observed was their marked decrease which lasted at all subsequent observation terms. Protein- hydropic degeneration and activation of hepatic macrophages remained for the longest period, but their level was lower compared with group I.

Fig. 14 shows dynamics of intralobular and portal infiltration intensity in the liver of animals from groups I and II. High intensity of hepatitis manifestations in on the 1st day of the experiment in group I and on the 3rd day in group II is quite noticeable. It agrees with maximum virus concentration in the lungs of animals which does not exclude maximum virus concentration in other organs. At later stages the process development was slight wavelike. In group II it was accompanied with much more considerable decrease in inflammatory alterations intensity in the liver of animals administered MFPC «Grinization».

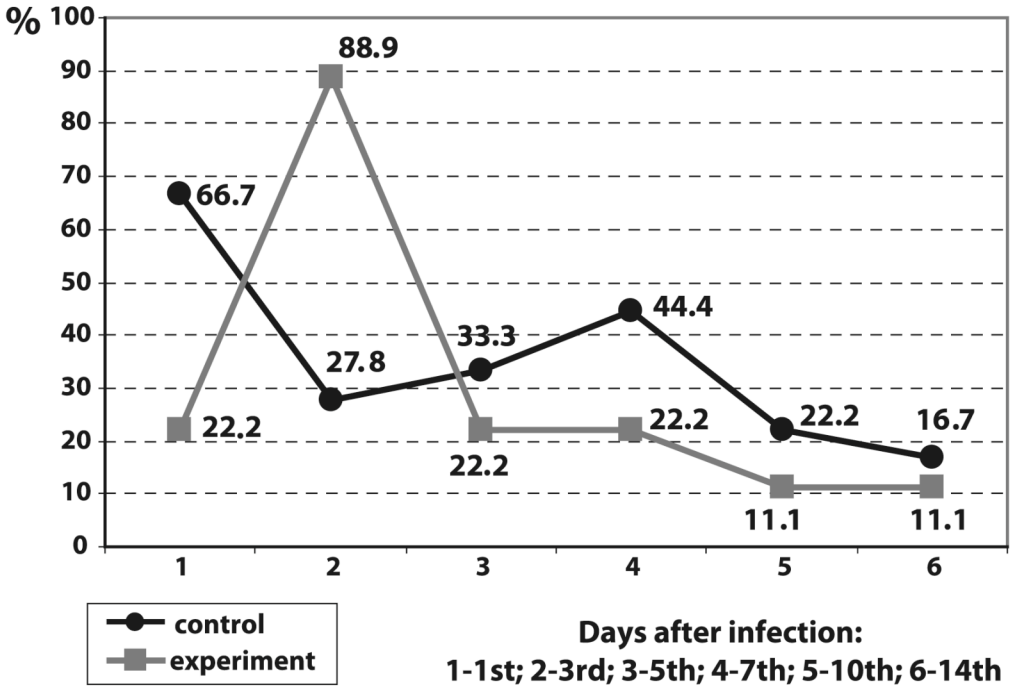


Fig.14. Dynamics of inflammatory infiltration development in the liver of animals from the group of control and the animals which were administered MFPC «Grinization».

Fig. 15 presents generalized characteristics (with account of all listed above signs) of changes in the liver of animals from group I and II.

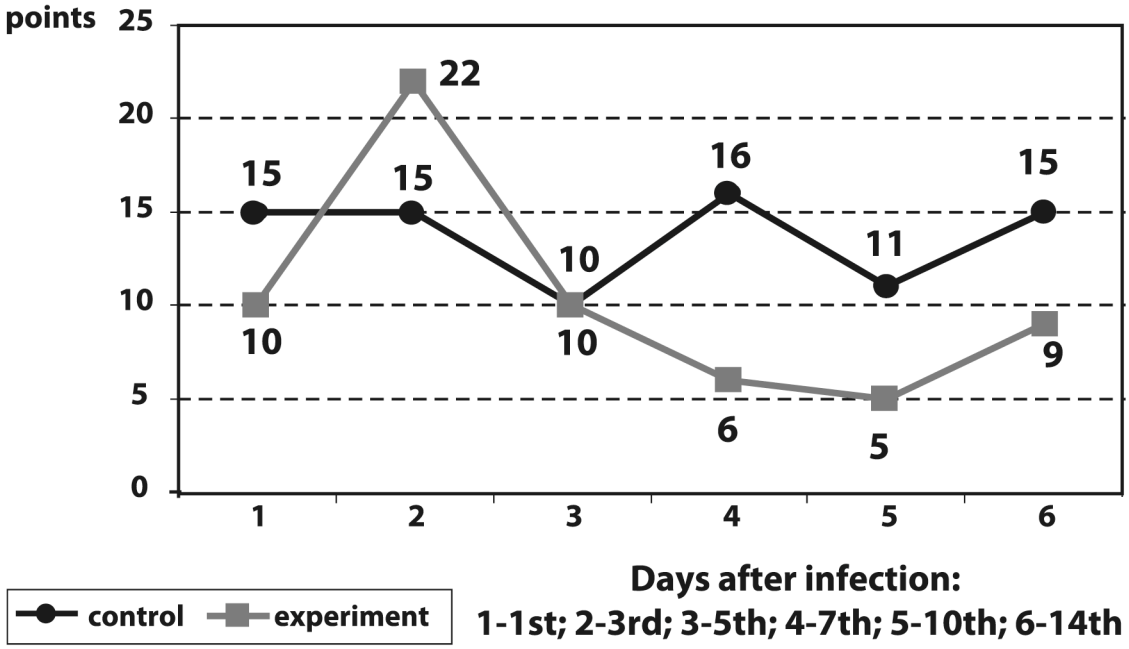


Fig. 15. General dynamics of inflammatory changes in the liver of animals from the group of control and the animals administered MFPC «Grinization».

In group I all the complex of morphological changes in the liver remained at the same level throughout the whole experiment with slight wave-like fluctuations within unreliable differences. In

group II the lesion volume was much more reduced on whole with maximum inflammatory alterations on the 3rd (Fig. 16).

Thus, influenza virus A/PR/8/34(H1N1) is present in the liver of infected mice of both groups and is eliminated by the end of experiment. Infected animals demonstrate persistent changes in the liver which can be estimated as viral hepatitis manifestations. In spite of influenza virus elimination degenerative changes of hepatocytes and proliferation of hepatic macrophages remain for the longest period of time in the group of control. Application of MFPC «Grinization» has a positive effect on development of pathologic changes in the liver and considerably reduces their intensity.

Alterations in the heart and kidneys of influenza virus infected animals had double nature. On one hand, they were changes conditioned by intoxication, hypoxia and haemodynamic compromise observed in all animals from group I. In the heart they manifested themselves by vessels congestions, focal degeneration and fragmentation of muscle fibres; in the kidneys there were also found congestive changes and focal degeneration of tubular epithelium. Tubulonecrosis was found in some animals from group I. Such changes were also present in animals from group II but they were much less intensive.

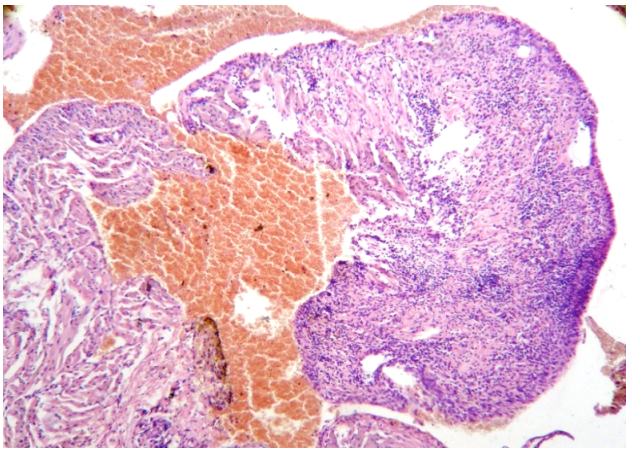


Fig.16. Acute endocarditis in virus A/PR/8/34(H1N1) infected animal, the third day. Hematoxilin-eosin staining, magnification × 200.

Other changes had inflammatory nature and in the heart they manifested themselves by single small infiltrates in the myocardium (viral myocarditis) in certain animals they were represented by endocarditis and pericarditis; glomerulonephritis manifestations and small-focal interstitial infiltration were present in the kidneys. Dynamics of the changes was similar to the one described in the liver. On the 1st day inflammatory changes in the heart and kidneys were absent then beginning with the 3rd day their intensity was growing with term observation increase and on the 10th -14th days 50% of the animals showed marked myocarditis and endocarditis as well as suppurative nephritis. In group II inflammatory changes in those organs were detected only on the 1st day and

later only slight degenerative changes were found. Thus, in A/PR/8/34(H1N1) infected animals there were found marked and persistent inflammatory and degenerative changes in the heart and kidneys which were much less intensive or even absent in the animals administered MFPC «Grinization».

5. CONCLUSIONS

MFPC «Grinization» possesses marked anti-influenza action which is demonstrated by considerable inhibition of viral reproduction and reduction in inflammatory changes intensity in the lungs and other organs. MFPC «Grinization» reduces risk of bacterial complications, significantly facilitates influenza course and decreases the risk of adverse outcome.

The list of mechanisms of MFPC «Grinization» action should include activization of systemic histiomacrophages reaction which is the feature of innate immunity and promotes efficient antiviral protection, limits viral infection spreading**.**

Activation of systemic proliferative processes in the lymphoid tissue is other meaningful mechanism of MFPC «Grinization» action. MFPC «Grinization» harmonizes immune response contributing to development of maximum immunity reaction on terms of maximum concentration of the virus and bacterial flora in the lungs and other organs.

Reduction of hepatitis manifestations in response to application of MFPC «Grinization» and normalization of detoxicating capacity of the liver improve the disease course considerably as well as belong to mechanisms of MFPC «Grinization» curative effect.

Thus, represented data provide strong evidence that MFPC «Grinization» reasonably to administer in a complex therapy and for the purpose of prophylaxis such actual infections as influenza and viral hepatitis, a and also for the prevention of progression of complications of these diseases.

We consider that using of the complex «Grinization» - it is the system of improving from a health point of view, based on the optimization of the cellular nutrition that is accompanied by improvement or normalization of metabolism. This leads to the relief of the alleviation of disease course or cure chronic infections and non-infectious pathology. In acute cases the administration of the complex «Grinization» significantly reduces the severity of the diseases courses and prevents processes of their chronization.

Use of the «Grinization» complex improves life quality of patients.

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