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THE INFLUENCE OF MULTIOPROBIOTICS «SYMBITER® ACIDOPHILIC» CONCENTRATED ON CHANGES IN RATS' LYMPHOID ORGANS UNDER PROLONGED DECLINE IN GASTRIC SECRETION OF HYDROCHLORIC ACID

It was investigated the reaction of thymus and spleen in rats with 28 days decrease of gastric acid secretion on injection of multiprobiotic «Symbiter® acidophilic» concentrated (SYM).

In rats, whom were injected only multiprobiotic SYM, relative thymus weight was not significantly changed in contrast to the relative cellness of this body, which grew significantly compared with the control. Hypoacid condition of gastric juice caused by the 28-day administration of rats by omeprazole (OM), led to a decrease of relative thymus mass while increasing relative content of lymphoid cells in this organ compared with the control group of animals. Putting away with OM multiprobiotic SYM caused growth of relative thymus weight compared with the group of rats who were administered OM, but the values of the control group animals and rats injected only SYM not reached. Relative cellness thymus in this group was in 2 times higher than in the control group, on 30% higher compared with rats with long hypoacid state and on 43% compared with rats, introduced by which only the SYM.

Putting animals multiprobiotic SYM did not significantly alter the relative weight of the spleen and significantly increased relative cellness compared with the control. 28 daily inhibition of gastric secretion of hydrochloric acid by OM in rats led to moderate splenomegaly: increased mass index and relative content of spleen lymphoid cells in this body as compared to control animals. Concomitant administration of OM multiprobiotic SYM did not significantly alter the relative weight of the spleen, compared with a group of long hypoacidity and a group of rats that received only SYM, and leave increased this figure compared with the control. Relative spleen cellness in the same group of rats, which injected with OM and SYM, was on 40% more ten in groups of animals that received only OM, on 112% than in the control group and not significantly different from the group of rats which was injected only SYM.

Thus, it was shown that long-term hypoacidity of gastric juice evoked cytomorphological changes in thymus and spleen. Injection of multiprobiotic «Symbiter® acidophilic» exert immunomodulatory action via activation of proliferative processes in observable lymphoid organs.

Key words: thymus, spleen, multiprobiotic, hypoacidity.

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Introduction. Reduced secretion of hydrochloric acid in the stomach enhances digestive tract colonization by various microorganisms as acidic environment is one of the most important factors of non-specific defense against bacterial infection [17, 29]. It is known that the microflora of the gastrointestinal tract performs immunomodulatory function at different levels of immune protection, supports immune homeostasis, actively interacting with the immune cells of the digestive tract, determine their differentiation affects the balance in the system of Th1 / Th2 and the synthesis of immune cells of many cytokines [11, 27]. There are appropriate of a prevention and a treatment of dysbiosis probiotics under conditions of the long hypoacidity of gastric juices.

Continuous reduction of gastric acidity caused by the introduction of proton pump inhibitors – omeprazole leads to morphological and functional changes in the gastrointestinal tract, inflammation and significant increase in gastrin in the blood (hypergastrinemia) [8, 34]. It is found that hiperhastrynemy is a risk factor for cancer of the stomach and colon [28, 30].

The negative effects of hypoacid of the gastric juice certainly affect in the immune system, which by many complex immune responses supports the physiological state of the body. Today special attention of researchers associated with ascertaining the regulatory role of various cytokines, which control certain immune responses, including those that significantly affect the functioning of the digestive tract. It is known that inflammatory cytokines IL-1 β and TNF- α are powerful factors inhibiting gastric secretion [9], and IFN- γ stimulates the production of G-cells of the stomach gastrin [33].

Modern experimental works devoted to studying the mechanisms of action of probiotics and their role in correcting violations that occur under prolonged oppression of gastric secretion of hydrochloric acid. Mechanisms of influence hypoacid state of the immune system and possible immunomodulatory properties multiprobitics under these conditions today are not agreed and cause interest in researchers.

The aim of the study was to explore cytomorphological reaction of thymus and rats' spleen introduced multiprobitic «Symbiter® acidophilic concentrated» under conditions of prolonged oppression of gastric hydrochloric acid secretion that was induced by administration of omeprazole.

Materials and methods. Research was conducted on nonlinear white male rats weighing 160-180 grams who were divided into four groups of 10 animals each. Manipulation with animals and their maintenance in vivarium carried out in accordance with international recommendations and national law to conduct biomedical research [4].

Control (I group) served as rats, which were injected intraperitoneally 0,2 ml (w/v) and 0,5 ml oral water for injection during the 28 days. The second group of rats was administered orally multiprobitic «Symbiter® acidophilic» concentrated (SYM) (produced by «O.D. Prolisok», Ukraine) at a dose of 0,14 ml/kg dissolved in 0,5 ml of water for injection.

Hypoacid condition in rats (group III) modeled daily administration for 28 days of omeprazole (OM) (production «Sigma-Aldrich», USA), that is a blocker of H^+/K^+ -ATP-ase – a key enzyme synthesis of hydrochloric acid gastric parietal cells. OM injected intraperitoneally once daily at a dose of 14 mg/kg, which was dissolved in 0,2 ml of water for injection. Rats fourth group simultaneously with the introduction of OM was injected multyprobiotic SYM, that is a living biomass concentrated symbiosis 14 unique probiotic strains of bifidobacteria, lactobacilli, propionic acid bacteria, lactococcus and physiologically useful products of their metabolism. It contains at least 10^9 living cells in 10 ml of SYM. The day before the experiment, the animals had access only to water .

The reaction lymphoid organs was assessed by weight indexes and relative content of lymphoid cells [21], which was calculated by determining the ratio of body weight to the total weight of the animal and the number of cells to body weight, respectively.

Rats were sacrificed by dislocation method of the cervical vertebrae day after the last injection, pre-weighed on electronic scales, removed thymus and spleen, which are also weighed and placed in a Petri dish with cold medium 199 («Sigma-Aldrich», USA). The cell suspension of lymphocytes obtained from thymus and spleen by providing on density gradient Ficoll-Paque («Sigma-Aldrich», USA) method [13]. Counting lymphoid cells with parallel determination of their viability by trypan blue staining was performed according to the method [5] in the Goryaev's chamber.

Statistical analysis of the results of studies using Student's t test was performed to assess the reliability of using Statistica 7.0. Differences considered significant at $r \leq 0,05$.

Results and discussion. Mass and cellness of lymphoid organs are integral indicators of generalized immune response. It should be informative studies provided only while calculating both indices since the change in mass of lymphoid organ can occur not only due to lymphoid cells, but also, for example, by epithelial cells or adipose tissue [2, 14].

Thymus is one of the key bodies in the development limphoyid organs in development of immune response, it's main function is the maturation of T-lymphocytes [35]. In addition, the thymus regulates the level of cellular and humoral immunity by export to the periphery of effector regulatory cells and also the biologically active mediators [31].

In the control rats group relative weight and relative thymus cellness amounted to $25 \pm 2,2 \times 10^{-4}$ conventional units and $60 \pm 5,5 \times 10^7$ conventional units (*fig. 1*). In rats, whom were injected only multyprobiotic SYM, relative thymus weight was not significantly changed and was $21 \pm 1,9 \times 10^{-4}$ conventional units in contrast to the relative cellness of this body, which grew significantly to $84 \pm 7,4 \times 10^7$ conventional units (40%, $p \leq 0,05$) compared with the control. Established effect may be related to a natural reaction to the administration, though useful, but of alien organisms consisting multyprobiotic, because it is known that probiotic microorganisms possess immunomodulatory properties specific abilites to incur an immune response, including the activation of T-dependent link immunity [12, 16, 23, 24].

Hypoacid condition called gastric juice daily administration of 28 rats OM led to a decrease relative thymus mass of $25 \pm 2,2 \times 10^{-4}$ to $15 \pm 1,2 \times 10^{-4}$ conventional units (32%, $p \leq 0,05$) while increasing from $60 \pm 5,5 \times 10^7$ to $92 \pm 8,3 \times 10^7$ conventional units (53%, $p \leq 0,05$) relative content of lymphoid cells in the body compared with the control group of animals.

Activation of proliferative processes in the rats thymus under long hypoacidity of the gastric juice, probably associated with the development of cell-mediated immune response and the need to attract to it an additional pool of T-lymphocytes. There is also evidence that there are characterized thymic hormones [6] in gastrin molecule fragments and they can stimulate immunogenesis [10].

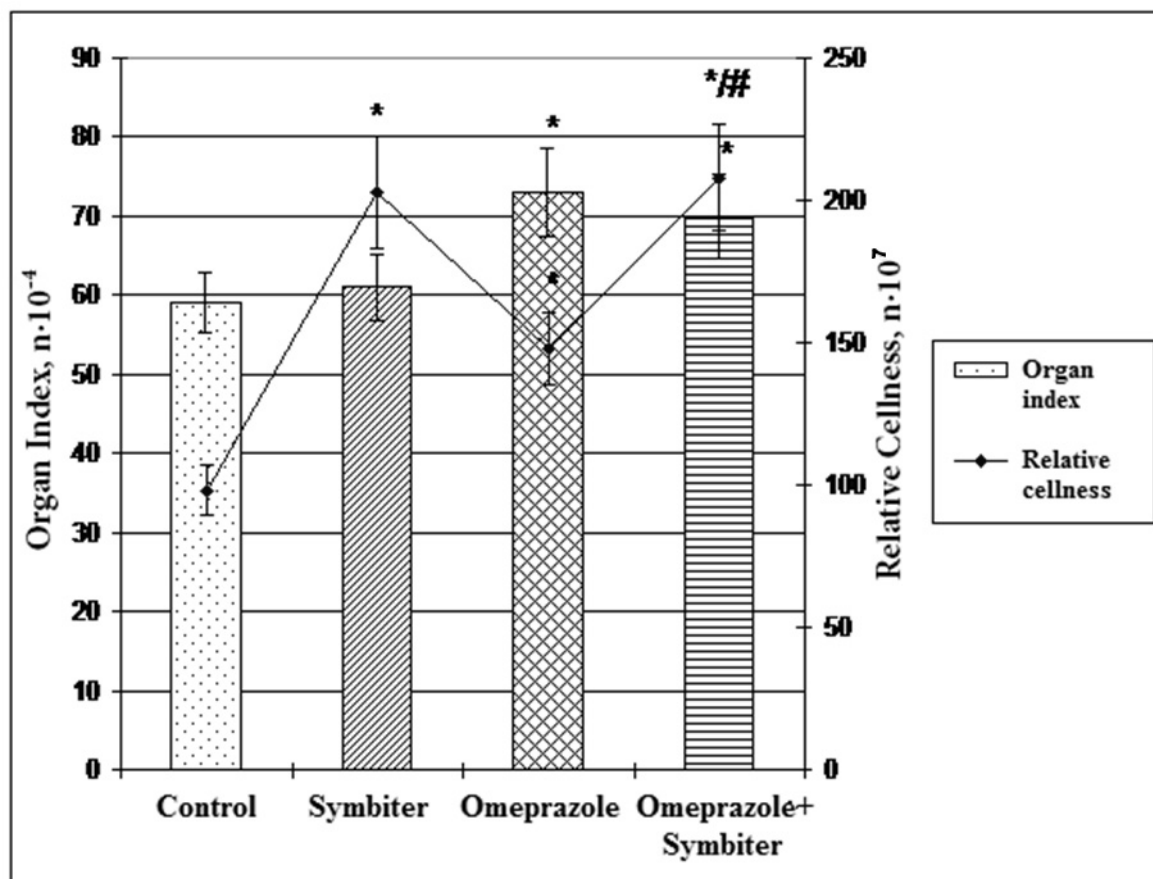


Fig 1. Cytomorphological rats' thymus condition with long hypoacid as the conditions of entry multiprobiotic ($M \pm n$, $n = 10$).

* – $P \leq 0,05$ compared to control;

– $P \leq 0,05$ compared with a group of animals which were administered omeprazole;

^ – $P \leq 0,05$ compared to the group of animals injected Symbiter.

Therefore, strengthening the proliferation in the thymus during prolonged inhibition of gastric secretion of hydrochloric acid can occur due to trophic action of gastrin, which concentration significantly increases at the 28-day administration of OM [3, 20]. It is known that the thymus is the most sensitive to the effects of chemical and physical factors [1]. Nevertheless, thymus degradation can occur not only as a result of toxic effects of OM, but also by inhibiting migration of stromal cells from bone marrow in the anemia and the need to constantly exports to the periphery of effector and regulatory cells from the thymus to attract immune response. It is known that anemia of chronic inflammation and the development of

dysbiosis is one of the main negative effects of prolonged hypoacidity of gastric juice [7, 18, 22, 26]. The results correlate well with the literature about the development of atrophy of the thymus in animals in applying the unique OM – lansoprazole and thymoprazole [25, 32].

Putting away with OM multiprobiotic SYM caused growth relative thymus weight by 18% ($p \leq 0,05$) compared with the group of rats who were administered OM, but the values of the control group animals and rats injected only SYM not reached and was $18 \pm 1,6 \times 10^{-4}$ conditional units. Relative cellness thymus in this group was $120 \pm 10,1 \times 10^7$ conditional units and was 2 times ($p \leq 0,05$) higher than the control group, 30% ($p \leq 0,05$) higher compared with rats with long hypoacid state and 43% ($p \leq 0,05$) compared with rats, introduced by which only the SYM. This cytomorphological reaction of thymus to enter the SYM under the hypoacid condition may be associated with immunomodulatory properties of probiotic microorganisms that cause activation of proliferative processes in the thymus to attract new T-cells to «fight» with inflammation and dysbiosis developing against the background of a prolonged hypoacid of gastric juices caused by omeprazole.

Sensibilizate antigen lymphoid cells migrate to secondary lymphoid organs, including the spleen. Microenvironment of the spleen facilitates intercellular contacts and generate an immune response. The main events that occur in the spleen, are the induction of T-dependent B-cell immune response generation of B-lymphocyte that produce antibodies, and proliferation of CD8 + T-lymphocytes. All this time the lien is in a transient splenomegaly rate, which is proportional to the level of activation of the immune response. In addition, the spleen plays an important role as a filtering organ (on by hematogenous spread antigen) and organ destruction of erythrocytes and platelets. Immune reactions in the body, leading to significant morphological changes in spleen [19].

The relative weight and relative spleen cellness control group of rats was respectively $59 \pm 3,8 \times 10^{-4}$ conditional units and $98 \pm 8,7 \times 10^6$ conditional unit (*fig. 2*).

Putting animals multiprobiotic SYM did not significantly alter the relative weight of the spleen, which was $61 \pm 4,2 \times 10^{-4}$ conditional units, and significantly increased relative cellness to $203 \pm 19,8 \times 10^6$ conditional units (107%, $p \leq 0,05$) compared with the control. Observed us strengthening of proliferation of lymphoid spleen cells may be associated with activation of microorganisms SYM not only cellular immunity, and humoral response to the phagocytic cells that presented antigens multiprobiotic.

28 daily inhibition of gastric secretion of hydrochloric acid OM in rats led to moderate splenomegaly: increased at under $73 \pm 5,5 \times 10^{-4}$ conditional units (24%, $p \leq 0,05$) and $148 \pm 12,7 \times 10^6$ conditional units (51%, $p \leq 0,05$) mass index and relative content of spleen lymphoid cells in this body as compared to control animals. This hypertrophic reaction of spleen, probably related, as with enhanced performance and phagocytic immune function, aimed at the elimination of foreign antigens during dysbiosis and to the performance of the tool «hemocateresis» the destruction of erythrocytes as a result of iron deficiency [15] and vitamin deficiency of B12 [18, 26] in rats with long hypoacidity of gastric juice.

Concomitant administration of OM multiprobiotic SYM did not significantly alter the relative weight of the spleen, which was $70 \pm 5,3 \times 10^{-4}$ conditional units ($p \leq 0,05$), compared with a group of long hypoacidity and a group of rats that received only SYM, and leave increased by 19% ($p \leq 0,05$) this figure compared with the control. Relative spleen cellness in

the same group of rats which injected with OM SYM was $208 \pm 18,5 \times 10^6$ conditional units ($p \leq 0,05$), which is 40% ($p \leq 0,05$) more groups of animals that received only OM 112% ($p \leq 0,05$) than the control group and not significantly different from the group of rats which was injected only SYM. The results may indicate a strengthening expansion of immune cells to the development of proliferative processes in the spleen as a result of immunomodulatory action SYM multiprobiotic the conditions hypoacidity of gastric juice in rats.

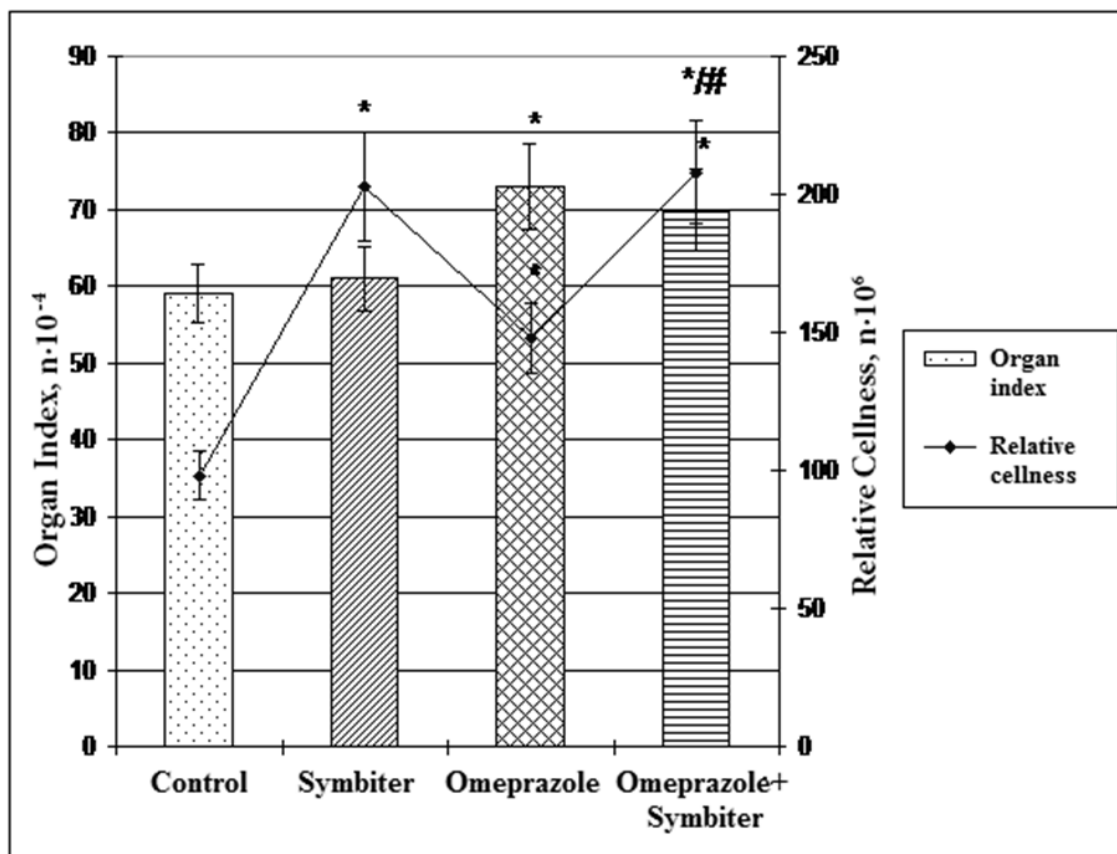


Fig 2. Cytomorphological state of rats spleen with long hypoacid state as the conditions of entry multiprobiotic ($M \pm n$, $n = 10$).

* – $P \leq 0,05$ compared to control;

– $P \leq 0,05$ compared with a group of animals which were administered omeprazole.

Conclusions. Long-term suppression of gastric secretion of hydrochloric acid leads to homeostatic alterations in the thymus and spleen of rats, that are likely related to the development of anemia, inflammation and dysbiosis in animals. Multiprobiotics SYM causes activation of proliferative processes in the studied lymphoid organs, which may be a manifestation of this immunomodulatory action involving various links of immunity to overcome the negative effects of prolonged hypoacidity of gastric juice in rats.

The obtained results give reason to consider that immunocompetent cells are involved in the immune response under conditions of hypoacidity of gastric juice. Elucidation of the mechanisms of immunomodulatory action of multiprobiotics «Symbiter® acidophilic»

concentrated facilitate its introduction into clinical practice of treating acid-associated diseases to overcome the negative effects of long-term reduction of gastric secretion of hydrochloric acid.

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ВЛИЯНИЕ МУЛЬТИПРОБИОТИКА «СИМБИТЕР® АЦИДОФИЛЬНЫЙ» КОНЦЕНТРИРОВАННЫЙ НА ИЗМЕНЕНИЯ В ЛИМФОИДНЫХ ОРГАНАХ КРЫС В УСЛОВИЯХ ДЛИТЕЛЬНОГО СНИЖЕНИЯ ЖЕЛУДОЧНОЙ СЕКРЕЦИИ СОЛЯНОЙ КИСЛОТЫ

Исследована реакция тимуса и селезенки крыс с 28-суточным снижением секреции соляной кислоты на введение мультипробиотика «Симбитер® ацидофильный» концентрированный (СИМ).

У крыс, которым вводили только мультипробиотик СИМ, относительный вес тимуса достоверно не изменялся, в отличие от относительного количества клеток этого органа, которое достоверно увеличивалось по сравнению с контролем. Гипоацидное состояние желудочного сока, вызванное 28-суточным введением крысам омепразола (ОМ), приводило к уменьшению относительной массы тимуса с одновременным увеличением относительного количества лимфоидных клеток в этом органе по сравнению с контрольной группой животных. Введение же вместе с ОМ мультипробиотика СИМ приводило к увеличению относительного веса тимуса по сравнению с группой крыс, которым вводили ОМ, но значений контрольной группы животных, которым вводили только СИМ, не достигало. Показатель относительной клеточности тимуса в этой группе был в два раза выше, чем в контрольной группе, на 30% выше по сравнению с крысами с длительным гипоацидным состоянием и на 43% больше по сравнению с крысами, которым вводили только СИМ.

Введение животным мультипробиотика СИМ достоверно не изменяло относительный вес селезенки и значительно увеличивало относительную клеточность по сравнению с контролем. 28-суточное угнетение желудочной секреции соляной кислоты с помощью ОМ у крыс приводило к умеренной спленомегалии: увеличивались массовый индекс селезенки и относительное содержание лимфоидных клеток в этом органе по сравнению с контрольными животными. Одновременное введение с ОМ мультипробиотика СИМ достоверно не изменяло относительный вес селезенки, по сравнению с группой с длительной гипоацидностью и группой крыс, которые получали только СИМ, и оставляло повышенным этот показатель по сравнению с контролем. Относительное же количество клеток селезенки в группе крыс, которым с ОМ вводили СИМ, была на 40% больше группы животных, которые получали только ОМ, на 112% больше контрольной группы и достоверно не отличалась от группы крыс, которым вводили только СИМ.

Таким образом, длительная гипоацидность желудочного сока вызывает цитоморфологические изменения в тимусе и селезенке крыс. Введение мультипробиотика оказывает иммуномодулирующее действие через активацию пролиферативных процессов в исследуемых лимфоидных органах.

Ключевые слова: тимус, селезенка, мультипробиотик, гипоацидность.

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ВПЛИВ МУЛЬТИПРОБИОТИКА «СИМБІТЕР® АЦИДОФІЛЬНИЙ» КОНЦЕНТРОВАНІЙ НА ЗМІНИ В ЛІМФОЇДНИХ ОРГАНАХ ЩУРІВ ЗА УМОВ ТРИВАЛОГО ЗНИЖЕННЯ ШЛУНКОВОЇ СЕКРЕЦІЇ СОЛЯНОЇ КИСЛОТИ

Досліджено реакцію тимусу й селезінки щурів із 28-добовим зниженням секреції соляної кислоти на введення мультипробиотика «Симбітер® ацидофільний» концентрований (СИМ).

У щурів, яким вводили тільки мультипробиотик СИМ, відносна вага тимусу вірогідно не змінювалася, на відміну від відносної кількості клітин цього органу, котра вірогідно збільшувалася порівняно із контролем. Гіпоацидний стан шлункового соку, спричинений 28-добовим введенням щурам омепразолу (ОМ), призводив до зменшення відносної маси тимусу з одночасним збільшенням відносної кількості лімфоїдних клітин у цьому органі у порівнянні з контрольною групою тварин. Введення ж разом із ОМ мультипробиотика СИМ призводило до збільшення відносної ваги тимусу у порівнянні із групою щурів, яким вводили ОМ, але значень контрольної групи тварин, яким вводили тільки СИМ, не досягало. Показник відносної клітинності тимусу у цій групі був удвічі вищим, ніж у контрольній групі, на 30% вище у порівнянні із щурами із тривалим гіпоацидним станом та на 43% більшим порівняно із щурами, яким вводили тільки СИМ.

Введення тваринам мультипробиотика СИМ вірогідно не змінювало відносної ваги селезінки та значно збільшувало відносну клітинність у порівнянні з контролем. 28-добове пригнічення шлункової секреції соляної кислоти за допомогою ОМ у щурів призводило до помірної спленомегалії: збільшувався масовий індекс селезінки та відносний вміст лімфоїдних клітин у цьому органі порівняно з контрольними тваринами. Одночасне введення з ОМ мультипробиотика СИМ вірогідно не змінювало відносної ваги селезінки у порівнянні із групою щурів, що мали тривалу гіпоацидність, та групою тварин, що отримували лише СИМ, і залишало підвищеним цей показник у порівнянні з контролем. Відносна ж кількість клітин селезінки у групі щурів, яким разом із ОМ вводили СИМ, була на 40% вище, ніж у групі тварин, котрі отримували лише ОМ, на 112% більше, ніж у контрольній групі тварин, і вірогідно не відрізнялася від групи щурів, котрим вводили тільки СИМ.

Таким чином, тривала гіпоацидність шлункового соку спричиняє цитоморфологічні зміни у тимусі та селезінці щурів. Введення мультипробиотика виявляє імуномодельюючу дію через активацию проліферативних процесів у досліджуваних лімфоїдних органах.

Ключові слова: тимус, селезінка, мультипробиотик, гіпоацидність.