Influence of A Genetically Modified Organism on The Rat's Hepatobiliary System

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Abstract: Introduction: In the experimental group of laboratory animals, visible changes in the spleen are noted, characterized by an increase in size, average weight, and changes in the structure and color of the organ under study. High synchronicity in quantitative changes in the cytoarchitectonics of the subcapsular, cortical and medullary substance of the thymus and the central and peripheral part of the lymphoid nodule and the red pulp of the spleen was revealed under the conditions of GMO use. It is characterized by inter-organ linear correlation relationships between cell types of the same name and different names, and in some cases even corresponds to the values within the organs. This means that a genetically modified product-soy flour - negatively affects the condition of the spleen and thymus.

Keywords: genetically modified product, laboratory animals, spleen, thymus, morphology.

Introduction

At the moment, much attention is paid to the problems of using genetically modified organisms (GMO) in food, since there is a threat of their negative impact on human health and the environment [5]. Genetically modified (transgenic) organisms are plants, animals, and micro-organisms whose genome has been altered by genetic engineering [6]. Gene technologies are increasingly being introduced into agriculture and the food industry. Changes in the DNA of plants and animals can affect the body in different ways.

Experts dealing with genetic security issues identify three types of threats that GMO pose: threats to the human body (in the form of allergic diseases, metabolic disorders, etc.), threats to the environment (in the form of vegetating weeds, chemical pollution, etc.), global risks (in the form of activation of critical viruses, threats to economic security) [7].

Scientists have realized that thanks to genetic modification, plants and vegetables become more frost-resistant, are stored longer, and are not eaten by insects.

By definition, GMO are living organisms that have intentionally altered nucleic acid sequences. These changes can be reduced to the introduction or removal of genetic fragments. In this case, both foreign nucleic acid (for example, bacteria containing the human insulin gene) and nucleic acid of this type can be introduced (for example, to increase the starch content in potatoes, the genes associated with starch synthesis can be "duplicated" several times). GMO combine three groups of organisms -genetically modified micro-organisms (GMO), animals (GMO), and plants (GMO). The dominant transgenic crops in the world are soy, corn, cotton, and rapeseed [11].

Creating genetically modified food sources is an inevitable way to solve many nutrition and health problems. Modern man consumes about 800 grams of food and 2 liters of water per day, the population of the planet – more than 400 million tons of food. There is a clear understanding in the international scientific community that due to the growth of the World's population, which, according to scientists ' forecasts, should reach 11 billion people by 2050 (2009 – more than 6 billion), respectively, there is a need to significantly increase world agricultural production, which is impossible without the creation of genetically modified organisms [9].

Genetically modified organisms – an organism or several organisms, any non-cellular, single-celled or multicellular formations capable of reproducing or transmitting inherited genetic material, other than natural organisms, obtained using genetic engineering methods and containing genetic engineering material, including genes, their fragments or a combination of genes [14].

Genetic engineering is a set of techniques, methods, and technologies, including technologies for obtaining recombinant nucleic acids, for isolating genes from an organism, manipulating genes, and injecting them into other organisms. Genetic engineering is the "heiress" of traditionally conducted breeding works in the field of crop production and animal husbandry. At the same time, with desirable genes, the possibility of transmitting undesirable ones is not excluded, which makes it difficult to separate positive properties from harmful ones. The priority of genetic engineering is the speed and accuracy of obtaining the desired properties, the ability to track and control genetic changes and their consequences [8].

Currently, genetically modified plants are grown in 28 countries around the world, especially in the United States, Brazil, Argentina, India and Canada. The main crops are soy, potatoes, corn, sugar beets, tomatoes, pumpkin, rapeseed [2,10,13].

A genome is a complete set of genes in an organism. DNA (deoxyribonucleic acid) is the "encyclopedia of life", the only type of molecule capable of encoding genetic information [15].

The identification of non-declared genetically modified food sources, as well as GMO combinations, is relevant for ensuring the biological safety of food for the population [1, 3, 4, 12].

Work on genetic modification is carried out in three main areas: agricultural plants, animals and birds, and microorganisms.

Modification of agricultural crops is aimed at searching for properties that provide resistance to herbicides, insecticides, viruses, adverse environmental factors, increasing product yield, improving consumer properties, nutritional value, etc. [15].

Currently, most GM foods are classified as the second class of safety, given the presence in their composition of 1-2 proteins responsible for the manifestation of the desired feature, which distinguishes the transgenic product from the traditional one. The concept of compositional equivalence may become untenable in the near future due to the beginning of mass production of transgenic products with a modified composition. As ways to solve this problem, it is proposed to use such areas of science as genomics-determination of the structure and function of DNA; proteomics- determination of protein profile; metabolomics-determination of secondary metabolites[14].

The biomedical assessment of GM food includes the following areas. Compositional equivalence – protein (amino acid composition), fat (fatty acid composition), carbohydrate composition, mineral composition, vitamin content, specific components, biologically active substances, contaminants (natural, anthropogenic);

Chronic toxicity of GM food-integral indicators, biochemical indicators, hematological indicators, morphological studies, sensitive biomarkers: the activity of enzymes of the I and II phases of biotransformation of xenobiotics, the activity of enzymes of the antioxidant defense system, the content of lipid peroxidation products.

New technologies for obtaining transgenic farm animals and birds are associated with increased productivity, optimization of individual parts and tissues of carcasses, have a positive impact on the quality, physical, chemical and technological properties of meat. The specificity and orientation of integrated genes allows changing the structure and color of muscle tissue, pH, stiffness, moisture-retaining ability, degree and character of fat content, consistency, taste and aroma properties of meat after processing [14].

The aim of the work was to study and evaluate the effect of the GM product on the morphological parameters of the spleen and thymus of laboratory animals in the experiment.

MATERIALS AND METHODS

Commercial soy flour (soy flour No. 24) was used as a GM product. Experimental studies were conducted on white mongrel rats.

All laboratory animals were divided into 3 groups: the experimental group-animals that included soy flour No. 24 in the General Vivar diet (at a dose of 0.02-0.03 g per 1 rat weighing 160-180 g for 30 days (n=30); the control group - animals that received only a General Vivar diet, without soy flour No. 24 (n=30). Group 3-intact animals (n=30) that were kept in standard vivarium conditions.

As a GM product, the experiments will use soy grown in Kyrgyzstan and imported to our country only for research. The PCR method revealed the presence of the 35S+FMV promoter in the studied GM soy, which proves that the studied soy is a GM product. In ordinary soy, this promoter is not present.

All groups were formed at the same time. The laboratory animals involved in the experiment were representative by age, gender, weight, and conditions of keeping and feeding. After 30 days of feeding soy flour No. 24, groups of laboratory animals were killed in a humane way, then autopsies were performed. When killing and dissecting laboratory animals, the rules of biological safety and ethical principles of working with laboratory animals were observed.

To study the morphological parameters of the spleen and thymus, a macroscopic method (anatomical dissection) was used. Macroscopic studies of animals were carried out on the basis of the meeting of the ethical Committee of the Ministry of health of the Republic of Uzbekistan No. 4/17-1442 dated 21.09.2020. It was based on the provisions of the Helsinki Declaration of the World Medical Association of 1964, supplemented in 1975, 1983, 1989, 1996, 2000, 2002, 2004, 2008, 2013.

To study morphological parameters, research methods widely used in laboratory practice were used. After cutting the material, it was fixed in 10% buffered formalin, then washed in water and dehydrated in alcohols and compacted with benzene. Then they were poured into paraffin and prepared sections 4-6 microns thick, which were stained with hematoxylin and eosin. The sections were examined morphometrically using an eyepiece micrometer DN-107T/ Model CM001 CYAN cope (Belgium).

Mathematical processing was performed directly from the General data matrix "Excel 7.0 "using the capabilities of the program" STTGRAPH 5.1". the standard deviation and representativeness errors were determined. When organizing and conducting research, the principles of evidence-based medicine were observed.

THE RESULTS OF THE STUDY AND DISCUSSION

The parameters of the spleen of laboratory animals of the experimental and control groups also significantly differed. Comparative changes in the spleen were related to size, structure (looseness), and color (dull). The spleen in the main group is enlarged and is equal to 29.7±5.4, the average weight in the control group was 0.65±0.10, and in the main group 0.87±0.13. The relative weight of the spleen, g/100 g of body weight 0.35±0.04 in the control group compared to the main 0.48±0.03. Changes in structure and color were also observed in the main group.

If the animals of the control group did not have an increase, change in the structure and color of the spleen (Fig.1), then in the experimental groups these parameters were markedly different compared to the control (Fig.2, 3)

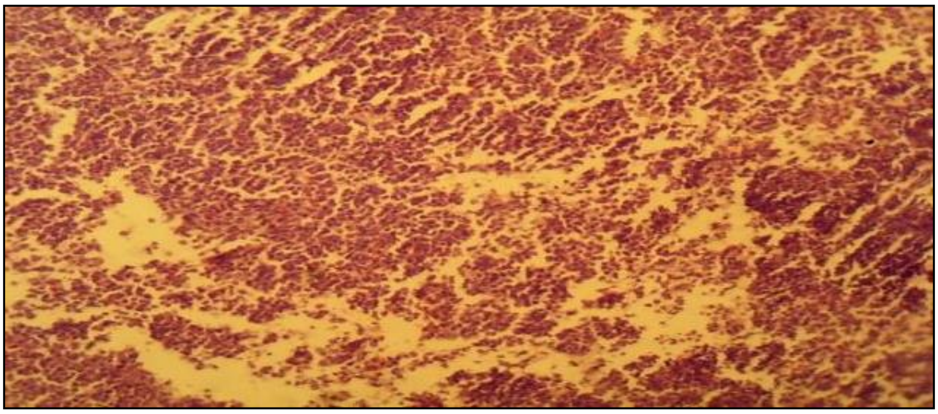

Fig.1. Spleen of a control group rat without pathological abnormalities (color by hematoxylin-eosin)

Changes in central and peripheral parts of the lymphoid nodules and red pulp of the spleen of mice after two weeks effects of GMOs indicate that a reduction in the number of small lymphocytes (1.3 – 2.5 times, $P<0.01$) and Mature plasma cells (more than 6 times, $P<0.01$) was observed with the increase in the number of cellular forms in a state of decomposition (1.8 - 4.57 times, $P<0.01$) and reticular cells is 2.5-5.3 times, $P<0.001$). At the same time, the content of less differentiated lymphocytes (blasts, large lymphocytes) does not significantly differ from the control. Increased destructive processes and a decrease in the number of differentiated lymphoid cells, plasma cells are signs of decompensation in the specified area of the spleen and indicate inhibition of b-lymphocyte differentiation processes.

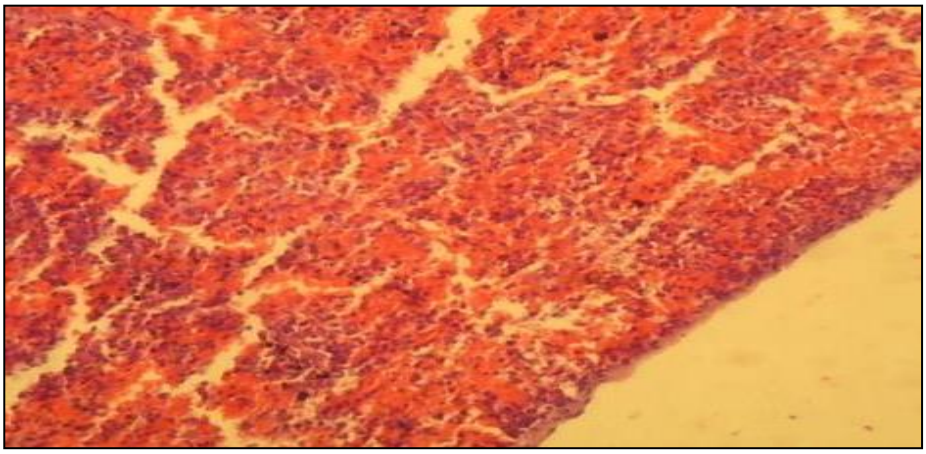

Fig.2. Hypertrophy, hyperplasia, edema, vascular fullness and spot hemorrhages of the spleen of the experimental group of rats (GMO soy flour No. 24, color by hematoxylin-eosin)

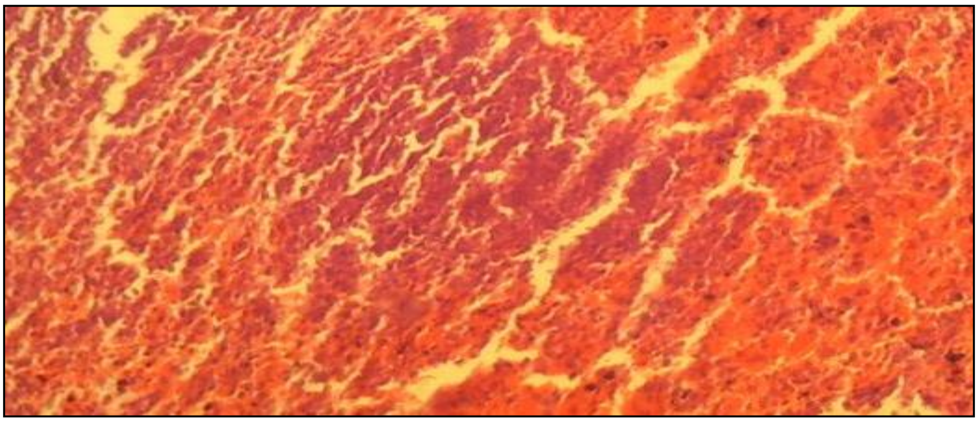

Fig. 3. Spleen: moderate swelling, moderate size increase (Non-GMO soy flour, color by hematoxylin-eosin).

In the thymus, after two weeks of GMO exposure, there were no sharp changes in the ratio of cortical and brain matter. To conclude about the features of the cytoarchitectonics of individual zones of the thymus, a quantitative study of the cytological profiles of the subcapsular, cortical, and medullary matter was performed. The absolute content of all encountered cell types per unit area of each structural and functional zone of the thymus was calculated. Significant deviations from the control (Fig.6) in the first two groups of rats (Fig.4,5) (a decrease in the number of small lymphocytes and the total number of all cells by more than 1.3 times, $P<0.01$) seem to indicate an increased functional activity of this zone, manifested in the active emigration of Mature differentiated forms of lymphocytes from the thymus. It should be noted that the increase in the number of mast cells (2.1 -3.7 times, $P<0.05$), detected in some cases, also indirectly indicates a change in the intensity of migration processes, since the role of mast cells in regulating blood vessel permeability is well known.

Fig. 4. Thymus: hypertrophy, hyperplasia of the thymus lymphoid tissue, congestive fullness, hemorrhages, signs of thymus depression and exhaustion. (experimental group) of GMO with soy flour No. 24 (color by hematoxylin-eosin)

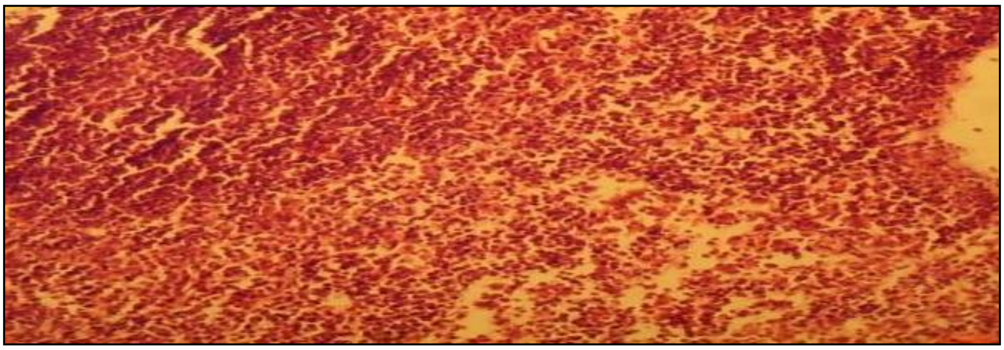

Fig.5. Thymus: full blood vessels, moderate hyperplasia of lymphoid tissue. Without GMO soy flour (experimental group) (color by hematoxylin-eosin)

Fig.6. Thymus: without pathological deviations, the standard food of the control group (color by hematoxylin-eosin).

Pathological changes in the spleen and thymus noted in the experimental group indicate that this GM product negatively affects the state of these organs in experimental animals. The absence of the carcinogenic effect of GM soy flour on the animals of the experimental group, apparently, was due to the short period of exposure to this food product.

CONCLUSIONS

In the experimental group of animals, visible changes in the spleen are noted, characterized by an increase in size, average weight, and changes in the structure and color of this organ. This means that the GM product-soy flour No. 24 has a negative effect on the condition of the spleen. In the spleen, the correlation of cytological profile indicators (absolute values that characterize the number of cells of different types), in contrast to the thymus, did not increase, but decreased. The maximum decrease in conjugation was found in the central part of the lymphoid nodule. This indicates a significant increase in cell autonomy and morphofunctional disorganization of individual areas of the organ, which makes it possible to attribute the spleen and, especially, its lymphoid nodules to the "weak links" of the immune system.

The effects of GMOs cause changes in the cytoarchitectonics of the subcapsular, cortical and medullary thymus, indicating increased immigration of t-lymphocyte precursors to the subcapsular zone, activation of t-lymphocyte differentiation stages in the thymus, and intensive emigration of medullary thymocytes against the background of a certain decrease in the level of destruction in the organ's medullary substance.

Significant disorganization of the cytological profiles of the subcapsular and cortical zones of the thymus was found, which indicates a greater vulnerability of these structures compared to the brain substance. In all areas of the thymus, the structure of the population of lymphoid cells changes most significantly, and in the subcapsular substance it is in an emergency state.

In the thymus, an increase in the correlation of cytological profile indicators (absolute values that characterize the number of cells of different types) was revealed): the maximum increase in conjugation was found in the brain substance, the minimum - in the subcapsular zone, which indicates an increase in the interaction of various types of cells in this lymphoid organ under the influence of GMOs, especially in its brain substance[7,8].

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