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Hematological-Biochemical and Morphological Indicators of Laboratory Animals under the Influence of Gene-Modified Soybean

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Abstract: The aim of the study was to laboratory animal's blood biochemical and hematological parameters, as well as the internal members to study the effects of the experiment. Experimental studies were carried out on white pedigree rats that were divided into experimental rats (soy flour No. 24, in a dose of 0.02-0.03 g per 1 rats for 30 days, n = 52) was included in the all-vegetable diet; Control group (only the general-purpose ration, n = 17). Found those laboratory animals, abattoirs and the experimental and control groups of different morphological parameters. In the blood of experimental animals studied biochemical and hematological indicators of imbalance. Thus, the GM-soybean adversely affects the liver, spleen, and certain biochemical and hematological indices of animal blood in the experiment.

Keywords: genetically modified product, laboratory animal experiment, biochemical and hematological parameters, morphology.

The term "genetically modified organisms" (GMOs) has appeared recently [1,5, 11]. Studies on the biomedical safety of GM products from potatoes, corn, soybeans, rice, cucumbers, tomatoes, sweet peppers, peas and rapeseed for food and feed production, carried out by independent scientists, and not by interested manufacturing companies, today there are very few.

Glyphosate-tolerant beans are currently the most widely used GM soybeans. It is about these varieties of GM soybeans that the results of the research are mainly available.

McCann et al. [3, 7,8] found that the nutrient composition of several varieties of commercial glyphosate-tolerant soybeans obtained after 3 years of breeding remained equivalent to that of conventional soybeans.

Kim S.H. et al. [6,9,12] found that the allergenicity of extracts obtained from common types of beans and GM soybeans was identical in the adult group of the studied people. [13,14] However, despite the positive results in the study of glyphosate-tolerant soy, other authors have concluded that more rigorous studies are needed to assess the allergenicity of GM soy and other GM foods, including a wide variety of controlled samples of GM soybeans [2, 4, 10].

The purpose of this study was to study and evaluate the effect of the GM product on the internal organs, biochemical and hematological parameters of the blood of laboratory animals in the experiment.

Materials and methods. Commercial soy flour (soy flour #24) was used as the GM product. Experimental studies were carried out on white outbred rats.

They were divided into 2 groups: the experimental group - animals that included soy flour No. 24 in the general diet (at a dose of 0.02-0.03 g per 1 rat weighing 160-180 g for 30 days (n=52); control group - animals that received only a general ration, without soy flour No. 24 (n=17).

Both groups were formed at the same time. The laboratory animals involved in the experiment were representative in terms of age, sex, weight, housing and feeding conditions. After 30 days of feeding No. 24 Soybean Meal, groups of laboratory animals were sacrificed humanely, followed by necropsy. When killing and autopsy of laboratory animals, the rules of biological safety and ethical principles of work with laboratory animals were observed [3, 6].

To study the morphological parameters of the internal organs, a macroscopic method (anatomical preparation) was used. Macroscopic studies of animals were carried out on the basis of SanPiN RUz No. 0185-05 [7]. To study hematological, biochemical parameters, research methods widely used in clinical and laboratory practice were used [1].

Statistical processing of the obtained results was carried out by conventional methods of variation statistics. When organizing and conducting research, the principles of evidence-based medicine were observed.

Research results and discussion. It should be noted that before the start of the main experiment, the rats of the experimental group were fed at an aggravated dose of 0.2-0.3 g per rat; but after 2 weeks of such a diet, the males of the experimental group began to visually observe edema and redness of the testicles, in connection with which the dose was reduced by 10 times.

A morphological examination of the internal organs showed that the heart of the experimental animals during visual examination was without visible changes, there were blood clots inside, the heart muscle was not hypertrophied, the dimensions were not enlarged, the color was normal. The state of the heart was almost identical in the experimental and control groups.

When examining the lungs, no visible pathological changes were found, both lungs were pink, the parenchyma remained unchanged, the structure of the lungs was intact, visual examination did not reveal tubercles, spots, or seals. This state of the lungs was visually observed in all animals of the experimental and control groups.

Macroscopic examination of the liver of animals revealed differences between the experimental and control groups. Thus, in 55.7±6.9% (n=29) of animals of the experimental group, an increase in liver size was found, while in the control group, the size remained within the normal range (Table 1).

Table 1 Indicators of macroscopic examination of the liver of experimental animals of the experimental and control groups

Indicators		Control group	Main group
The liver is enlarged, %		0	55,8±6,9*
Average weight of the liver, g		5,88±1,07	7,58±1,21
Relative weight of the liver, g/100 g of body weight		3,27±0,32	4,21±0,46*
Change in the structure of the liver, %		11,8±7,8	65,4±6,6*
Liver color change, %		5,8±5,6	7,7±3,7
The consistency of the liver,%	soft	0	9,6±4,1*
	dense	5,9±5,6	0

Note: * - reliability of differences in relation to control.

The differences between the compared groups were in the average weight of the liver, if the average weight of the liver in the experimental group was 7.58 ± 1.21 g, then in the control group this parameter was 5.88 ± 1.07 g, which is 1.3 times less than in the experimental group. A significant difference was observed in the relative weight of the liver (g/100 g of body weight) - P<0.05.

The difference between the compared groups also concerned such indicators as "changes in the structure of the liver" (P<0.001), that is, the liver had a heterogeneous, loose structure; "Change in the color of the liver" (P> 0.05), when the color of the organ was dull with a change from the natural shade.

The parameters of the spleen of laboratory animals of the experimental and control groups also differed significantly (Table 2). Comparative changes in the spleen were related to size, structure (friability) and color (dull).

Table 2 the results of macroscopic examination of the spleen of experimental animals of the experimental and control groups

Indicators	Control group	Main group
The spleen is enlarged, %	0	30,8±6,4*
Average weight of the spleen, g	0,63±0,12	0,90±0,10*
Relative weight of the spleen, g/100 g of body weight	0,35±0,04	0,50±0,05*
Structure change, %	0	7,7±3,7*
Color change, %	0	13,5±4,7*

*Note: * - reliability of differences in relation to control.*

If in animals of the control group there was no increase, change in the structure and color of the spleen, then in the experimental group these parameters differed markedly compared to the control.

Macroscopic examination of the stomach, small and large intestines in animals of the control group showed no visible pathological changes.

In the experimental group, visible changes were found in the structure of these organs and / or the presence of a putrid odor at autopsy, but these changes concerned a small number of experimental animals and the results of statistical analysis were not reliable. In addition, laboratory animals showed practically no signs of the formation of tumor processes. In all the studied organs, no visible tumor-like formations were found.

Thus, the GM product (soy flour No. 24) did not have a negative effect on the heart and lungs of laboratory animals when fed for 30 days at a dose of 0.02-0.03 g per 1 rat. At the same time, pathological changes in the liver and spleen noted in the experimental group indicate that this GM product negatively affects the state of these organs of experimental animals. The absence of a 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.

The obtained results of biochemical blood tests show that in animals of the experimental group there is a significant decrease in creatinine by 1.3 times (P<0.05), conjugated bilirubin by 1.7 times (P<0.001), AST by 1.5 times (P <0.02), ALT, urea and glucose, respectively, by 1.4 times (P<0.05) in relation to these parameters of the control group (Table 3).

It was found that the indicators of total protein were increased by 1.1 times in the animals of the experimental group in relation to the data of the control group. In addition, the free bilirubin index was 1.5 times higher than in the control (P<0.05).

The results of hematological analyzes show that in the animals of the experimental group, hemoglobin was reduced by 1.2 times in relation to the control data - 63.16±7.06 g/l and 75.66±8.46 g/l, respectively (Table 4). In addition, the number of leukocytes was reduced by 2.6 times in relation to the control - respectively 1.95±0.36 x 109/l and 5.05±3.59 x 109/l (P<0.001).

Table 3 Biochemical parameters of blood in white outbred rats depending on their feeding with soy flour No. 24, M±m

Indicators	Norm	Group	
		Control	Experience
Creatinine mg/dl	0.47-0.49	138,22±13,52	106,51±24,60 ↓*
Total protein, g/l	69	97,33±6,83	110,65±6,98 ↑*
Total bilirubin, mg/dL	1,7-3,2	38,04±1,51	37,62±4,39
Bound bilirubin, mm/l	0-5,1	20,54±1,62	11,79±3,25 ↓*
Free bilirubin, mm/l	15,0	17,49±0,84	25,83±2,30 ↑*
AST, E/l	40-58	40,36±1,29	26,28±3,13 ↓*
ALT, U/l	56-70	35,44±2,44	24,61±3,05 ↓*
Uric acid mg/dl	3,0-7,8	2,45±0,56	2,88±1,30
Cholesterol, mmol/l	1,8-1,9	1,68±0,61	1,65±0,45
Urea, mmol/l	7,1-8,3	52,97±15,15	36,81±11,00 ↓*
Glucose, mmol/l	4,2-4,4	5,67±0,88	4,05±0,70 ↓*

*Note: * - reliability of differences in relation to control; ↑, ↓ - increase or decrease of the indicator in relation to the control.*

At the same time, in the blood of animals of the experimental group, Jolly bodies appear in erythrocytes (1.9±1.3%) and toxic granularity of leukocytes (1.9±1.3%), which were not observed in the control group.

Thus, the study of blood biochemical parameters showed that in the animals of the experimental group there was an imbalance of indicators, such as an increase in total protein, free bilirubin, a significant decrease in conjugated bilirubin, ALT, AST, urea and glucose in relation to the control group. These data indicate tension in the hepato-biliary system of the experimental animals.

The studied hematological parameters showed that in the experimental group, hemoglobin decreases and the number of leukocytes significantly decreases, which indicates a decrease in the body's immune resistance. The appearance of toxic elements (Jolly bodies in erythrocytes and toxic granularity of leukocytes) indicates the presence of pathological processes in the body and a decrease in the antitoxic properties of the liver.

Table 4 Hematological parameters of blood in white outbred rats depending on feeding them with soy flour No. 24, M±m

Indicators	Norm	Group	
		Control	Experience
Hemoglobin, g/l	113-117	75,66±8,46	63,16±7,06 ↓*
Erythrocytes, 1012/l	7,9-8,5	3,30±0,24	2,86±0,24
Color indicator, units	1,0	0,66±0,03	0,65±0,02
Leukocytes, 109/l	7,0-7,3	5,05±3,59	1,95±0,36 ↓*
Stab neutrophils, %	1-6	4,16±1,22	3,33±0,49
Segmented neutrophils, %	47-72	62,83±6,50	66,16±4,31
Lymphocytes, %	63-66	24,0±4,53	24,16±3,30
Monocytes, %	4,1-5,1	7,5±2,55	4,5±1,23
ESR, mm/h	1,5-2,1	3,33±0,42	2,83±0,30
Myelocytes, %	-	0,33±0,33	0,33±0,21
Basophils, %	0-1	1,5±0,71	1,33±0,66
Jolly bodies in erythrocytes, %	-	0	0,66±0,49 ↑*
Toxic granularity of leukocytes, %	-	0	0,25±0,20 ↑*

*Note: * - reliability of differences in relation to control; ↑, ↓ - increase or decrease of the indicator in relation to the control.*

Conclusions.

1. In macroscopic examination of the liver of laboratory animals, different parameters were found between the experimental and control groups, which concerned the size of the liver, the average weight of the liver, "change in the structure of the liver" and "change in the color of the liver", where the data of the experimental group were significantly higher than the parameters of the control. This indicates that GM soy has a negative effect on the state of the liver in the dynamics of the experiment.
2. In the experimental group of animals, visible changes in the spleen are noted, characterized by an increase in size, average weight, changes in the structure and color of this organ. This means that GM soy, along with the liver, also negatively affects the condition of the spleen.
3. In the blood of animals of the experimental group, there is an imbalance of the studied biochemical parameters, characterized by an increase in total protein, free bilirubin, a significant decrease in conjugated bilirubin, ALT, AST, urea and glucose in relation to control. This indicates tension in the hepato-biliary system of the animal organism.
4. In laboratory animals of the experimental group, hemoglobin values decrease and the number of leukocytes significantly decreases, which indicates a decrease in the body's resistance. The appearance of toxic elements indicates the presence of pathological processes in the body and a decrease in the antitoxic properties of the liver.

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