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Subacute toxicity of the preparation "Biovir-P".

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ABSTRACT

The development and application of biologically active preparations based on peptidoglycans (PGs) of lactic acid bacteria is extremely important to livestock farming in modern conditions, since PGs are considered as natural nonspecific stimulants of immunogenesis which increase the body's resistance to adverse factors. The article presents data on the toxicity of the product Biovir-P made on the basis of PG lactic acid bacteria with long-term usage. Animals of the experimental groups received the product at a dose of I-12.5, II-25, III - 125 mg/kg of body weight. It has been established that the use of supplying white rats with a dose of 12.5 mg/kg of body weight during 14 days contributed to a possible increase in hemoglobin, the number of leukocytes and red blood cells by 9.2, 18.3 and 7.6% ($p < 0.05$) respectively compared to the control group. However, the 3rd experimental group of animals did not demonstrate a significant increase of the indicators mentioned above. Subsequently, the use of Biovir-P for the period of 28 days led to an increase in hematological parameters only for animals which were supplied with doses of 12.5 and 25 mg/kg body weight. In the rehabilitation period, on the 42nd day of the experiment in the 1st and 2nd experimental groups of animals, the hemoglobin concentration, the number of red blood cells and leukocytes remained high, while in the third experimental group of animals these rates were still lower than that of the control group. While estimating the content of total protein and its fractions in blood serum of experimental animals, it was found out that the use of the product over the course of 14 days contributed to the increase of the total protein concentration and the tendency to increase the level of albumin in animals of all experimental groups. On the 28th day of the experiment in the 1st and 2nd experimental groups of animals, the concentration of total protein and albumin was slightly higher than those of control animals, and these rates decreased for the animals of the third experimental group. The same trend was observed for the animals of the 1st and 2nd experimental groups during the rehabilitation period. The investigated product positively influenced the factors of nonspecific protection, in particular, it caused an increase in phagocytic activity of neutrophils and phagocytic index, with a slight decrease in the number of circulating immune complexes.

Keywords: subacute toxicity, peptidoglycans, biochemical parameters, non-specific resistance, phagocytic index.

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INTRODUCTION

The state of immunological resistance of an organism is one of the main etiopathogenetic factors of diseases of an infectious and non-infectious nature. Reducing the body's resistance combined with the emergence of antibiotic resistant strains of microorganisms, it leads to the spread of infectious diseases that are difficult to treat using the traditional methods of treatment. In such cases stimulation of the immune system by various biologically active agents is reasonable (1-5).

In view of this, special preparations of natural origin or their analogues deserve special attention. In this aspect, the use of peptidoglycans (PGs) of the microbial wall of lactic acid bacteria is extremely promising (6, 7).

Peptidoglycans are polymers consisting of sugar and amino acids that form a homogeneous layer around the plasma cell membrane of bacteria, thereby counteracting not only the osmotic pressure of the cytoplasm, but also playing the structural role of the cell wall, forming its shape and strength (8, 9).

The active substance of PG is a muramyl dipeptide, which is formed from normal intestinal microflora of men and animals under the influence of lysozyme and other lytic enzymes which are constantly present in the intestines. Penetrating into the blood, these compounds provide various neuro-and immunoregulation effects (10). This is primarily due to the fact that PG is characterized by adjuvant properties in both humoral and cellular responses (11). In addition, PGs and their derivatives are characterized by immunomodulatory activity (12) and therefore they should be considered as natural nonspecific stimulators for immunogenesis. However, today in the practice of veterinary medicine of Ukraine biologically active preparations of microbial origin have not become widely used.

The purpose of our work was to study the pharmaco-toxicological parameters of the drug "Biovir-P". To achieve this goal, we were faced with the following tasks: to study the dynamics of the effect of the preparation on the hematological, biochemical and non-specific resistance factors of the organism of laboratory animals for the long-term use of BioVirus-P and in the recovery period.

MATERIALS AND METHODS

To study the subacute toxicity of the drug "Biovir-P", a control and three experimental groups of white rats were formed, 18 animals per each one. Animals of the control group were administered with isotonic sodium chloride solution. For animals and experimental group, the preparation was administered at a dose of 12.5, II-25, III-125 mg/kg of body weight. The preparation was administered gastrointestinally with a probe for 28 days in the morning. On the 14^{th}, 28^{th} and 42^{nd} days of the experiment, under conditions of light etheric anesthesia, animals were decapitated and blood samples were taken for hematological and biochemical studies [13-15]. All studies were conducted in accordance with the principles of the "European Convention on the Protection of Vertebrate Animals used for Experimental and Scientific Purposes" and the Decree of the First National Congress on Bioethics (16, 17).

RESULTS AND DISCUSSION

The composition of the product under investigation includes a complex of activated low molecular weight peptides of the cell walls of lactic acid bacteria of the strains Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus fermentum, Bifidobacterium bifidum, Bifidobacterium longum.

For long-term administration of the drug, it is extremely important to establish the dynamics of the effects of various concentrations of the test substance on the organism of laboratory animals. Usage of Biovir-P for the period of 28 days and during the rehabilitation period led to the results provided in Table 1.

Table 1: Influence of Biovir-P on hematological indices of white rats (M ± m, n = 6)

Animal groups	Hemoglobin, g/l	Erythrocytes, T/l	Leucocytes, G/l
14 days			
Control	121,2±4,47	6,62±0,15	9,4±0,39

I experimental	132,3±2,17*	7,12±0,16*	11,1±0,53*
II experimental	133,3±2,19*	7,15±0,17*	11,3±0,35**
III experimental	127,4±2,14	6,60±0,23	9,70±0,41
28 days			
Control	122,3±3,09	6,82±0,09	10,2±0,29
I experimental	132,8±3,15*	7,30±0,15*	12,8±0,61**
II experimental	126,5±2,39	7,05±0,23	10,6±0,34
III experimental	110,7±2,79*	6,40±0,32	9,20±0,58
42 days			
Control	124,7±2,76	6,72±0,36	10,3±0,42
I experimental	126,2±2,14	6,98±0,19	11,1±0,56
II experimental	124,5±2,28	6,82±0,27	10,2±0,57
III experimental	118,4±3,22	6,60±0,29	9,40±0,50

Note: The degree of probability for the control group: * - p <0,05, ** - p <0.01.

As it can be seen from the data given in Table 1, the use of Biovir-P positively influenced the body of laboratory animals. In particular, on the 14th day of the product usage in the I experimental group of animals, there was a probable increase in the level of hemoglobin, the number of leukocytes and erythrocytes, by 9.2, 18.1 and 7.6% (p <0.05) respectively compared to the values of the control group. For the animals of the 2nd experimental group which were administered with the preparation at a dose of 25 mg/kg body weight hemoglobin concentration, the number of leukocytes and erythrocytes also increased significantly, by 9.9 (p <0.05), 20.2 (p <0,01) and 8% (p <0,05) respectively compared to the control group values. In the 3rd experimental group of animals, which were administered with a 10-fold dose, a slight increase was observed in the indicators mentioned above.

Subsequently, the use of Biovir-P during the 28 days caused the increase of hematological parameters only in the 1st and 2nd experimental groups of animals. Under these conditions, the concentration of hemoglobin, the number of white blood cells and red blood cells in blood of the animals of the 1st experimental group increased by 8.6 (p <0.05), 25.5 (p <0.01) and 7% (p <0 , 05) respectively. As for the indices noticed in the third experimental group of animals, it should be noted that they were lower than those of the control group animals.

In the rehabilitation period, on the 42nd day of the experiment, the hemoglobin concentration, the number of red blood cells and leukocytes remained high in blood of the animals of the 1st and 2nd experimental groups, whereas in the 3rd experimental group of animals, these indices were still lower than for the animals not only of control but the 1st and 2nd experimental groups.

Further on, the effect of the product under investigation on the content of total protein and its fractions in blood serum of the experimental animals was studied. The results of the studies are presented in Table 2.

Table 2: Influence of Biovir-P on the content of total protein and its fractions in serum of white rats blood (M ± m, n = 6)

Indices	Animal groups			
	Control	I experimental	II experimental	III experimental
14 days				
Total protein, g/l	69,4±2,06	77,5±2,60*	78,3±2,19*	79,2±2,37*
Albumin,%	42,4±0,75	44,3±0,58	44,8±0,88	45,2±1,04
α-1 globulins, %	8,8±0,32	7,7±0,64	7,4±0,64	8,4±0,55
α-2 globulins, %	11,1±0,95	9,5±0,54	9,6±0,43	9,3±0,40
β- globulins, %	19,5±0,77	17,9±0,47	17,4±0,52	17,6±0,47
γ- globulins, %	18,2±0,49	20,5±0,57*	20,8±0,68*	19,5±0,55
28 days				
Total protein, g/l	69,7±1,83	74,4±2,45	72,4±1,35	67,3±1,67
Albumin,%	41,7±0,52	42,3±0,77	41,9±0,75	41,2±0,73

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α-1 globulins, %	9,5±0,37	8,7±0,57	9,2±0,73	10,2±0,26
α-2 globulins, %	10,7±0,46	9,5±0,54	9,9±0,66	11,0±0,33
β- globulins, %	19,3±0,77	17,7±0,65	17,4±0,43	19,2±0,43
γ- globulins, %	18,6±0,56	21,7±0,42**	21,4±0,47**	18,2±0,53
42 days				
Total protein, g/l	71,2±2,02	71,9±2,50	70,8±1,48	69,7±1,69
Albumin,%	43,3±0,46	44,7±0,68	43,5±0,42	42,9±0,48
α-1 globulins, %	8,9±0,53	7,5±0,31	8,1±0,29	9,4±0,18
α-2 globulins, %	9,8±0,61	7,9±0,63	8,6±0,29	10,1±0,24
β- globulins, %	18,6±044	17,1±0,64	17,4±0,37	18,7±0,27
γ- globulins, %	19,3±0,76	22,7±0,41**	22,3±0,46**	18,8±0,44

Note: The degree of probability for the control group: * - p <0,05, ** - p <0.01.

As it can be seen from the data in Table 2, the use of the preparation for 14 days contributed to an increase in the concentration of total protein. In particular, in animal blood of groups I, II and III, the total protein concentration probably increased by 11.7, 12.8 and 14.1% respectively ($p < 0.05$). In the analysis of protein fractions in animals of the latter groups, there was a tendency to increase the quantity of albumins by 4.5, 5.7 and 6.6% respectively as compared to the control group. On the 28th day of the experiment with animals in groups I and II, the concentration of total protein and albumin was slightly higher than that of control animals. However, in the third experimental group of animals, these rates decreased. The same trend was observed with the animals of the 1st and 2nd experimental groups on the 42nd day of the experiment, the concentration of total protein and albumin remained higher than that of the control group animals, whereas the situation with the animals of the third group was different – the level was lower.

While evaluating the concentration of γ-globulins for the animals of the 1st and 2nd experimental groups on the 14th day of the experiment, the probability of growth of this index was determined by 12.6 and 14.2% respectively ($p <0.05$), and on the 28th day – by 16.7 and 15.1% ($p <0.01$). However, it is worth mentioning that the concentration of γ-globulins in the blood of animals of the third experimental group was lower by 2.2% than the control group values.

In the rehabilitation period, the level of γ-globulins in the 1st and 2nd experimental groups of animals remained high and was significantly higher at 17.6 and 15.5% ($p <0.01$), compared to the control group, whereas for animals in the third group this indicator was lower by 2.6%. Moreover, under these conditions, with the increase of γ-globulins in the animals of the 1st and 2nd experimental groups, a decrease in the levels of α-1, α-2 and β-globulins was observed, which is obviously due to the redistribution of protein fractions in favor of γ- globulins.

While studying the effect of Biovir-P on some biochemical parameters of blood serum and activity of enzymes, the data given in Table 3 were obtained.

Table 3: Biochemical parameters of blood serum of white rats (M ± m, n = 6)

Indices	Animal groups			
	Control	I experimental	II experimental	III experimental
14 days				
Bilirubin total, Mmol/l	3,78±0,45	3,76±0,69	3,74±0,42	3,70±0,64
Cholesterol total, Mmol/l	1,43±0,12	1,41±0,13	1,38±0,11	1,36±0,12
Urea, mol/l	7,58±0,57	7,42±0,42	7,63±0,48	7,57±0,45
Creatinine, µmol/l	74,3±1,61	74,4±1,93	74,8±1,89	74,5±1,43
AST, U/L	180,2±14,7	182,5±10,2	190,4±14,3	193,8±12,6
ALT, U/L	82,2±3,80	81,8±3,09	81,3±2,32	80,8±2,31
LF, U/L	156,3±12,5	158,2±14,5	150,8±11,6	146,8±10,7
28 days				
Bilirubin total, Mmol/l	3,83±0,68	3,54±0,77	3,48±0,53	3,72±0,63
Cholesterol total, Mmol/l	1,50±0,09	1,48±0,14	1,40±0,13	1,36±0,15
Urea, Mmol/l	7,85±0,40	7,88±0,56	7,57±0,45	7,12±0,68

Creatinine, Mmol/l	76,7±1,97	75,8±1,89	75,3±1,81	74,2±1,77
AST, U/L	182,5±10,3	179,4±11,9	182,4±14,2	172,4±9,92
ALT, U/L	80,6±3,67	80,3±3,41	78,9±3,28	78,2±3,57
LF, U/L	132,8±9,94	130,1±13,2	127,4±15,9	124,1±13,8
42 days				
Bilirubin total, Mmol/l	3,92±0,39	3,94±0,82	3,98±0,78	3,96±0,61
Cholesterol total, Mmol / l	1,38±0,14	1,33±0,11	1,36±0,13	1,37±0,14
Urea, mol / l	7,93±0,56	7,88±0,52	7,95±0,46	7,83±0,61
Creatinine, µmol / l	73,3±1,69	72,1±1,18	72,7±0,99	73,1±1,67
AST, U/L	179,4±9,24	178,9±10,1	172,7±10,9	175,2±13,8
ALT, U/L	78,2±4,97	77,6±2,16	82,3±1,98	78,9±3,03
LF, U/L	151,7±16,3	150,4±10,8	152,0±16,7	149,2±11,9

As the data presented in Table 3 demonstrate, the 14th day of the experiment showed a slight decrease in the concentration of total bilirubin and cholesterol in animals of all experimental groups. While evaluating the activity of blood serum enzymes in experimental animals, no significant changes were found in AST, ALT, and LF in the study period, the same trend was observed on the 28th day of the experiment. On the 42nd day of the experiment, in the 1st and 2nd experimental groups of animals the indices mentioned above were within the physiological norm, whereas for the animals of the third experimental group they were slightly lower than those of the control animals and the 1st and 2nd experimental groups.

It is extremely important to figure out the influence of biologically active preparations on the factors of non-specific immunity, so the next stage of our research was to study the effect of Biovir-P on factors of nonspecific protection. The results of the studies are presented in Table 4.

Table 4: Influence of Biovir-P on factors of non-specific protection of white rats (M ± m, n = 6)

Animal groups	Phagocytic activity neutrophils, %	Phagocytic index, mt/neutr	Circulating immune complexes, U/l
14 days			
Control	20,1±0,57	12,1±0,38	22,5±2,57
I experimental	21,7±0,41*	13,1±0,26	21,8±3,00
II experimental	21,8±0,39*	12,9±0,41	21,7±2,54
III experimental	20,4±0,43	12,6±0,34	22,7±2,94
28 days			
Control	20,2±0,62	12,6±0,48	20,5±3,05
I experimental	22,4±0,50*	13,6±0,76	18,8±3,19
II experimental	22,2±0,61*	13,4±0,64	19,7±2,40
III experimental	20,1±0,53	12,4±0,47	23,7±3,11
42 days			
Controls	20,5±0,90	13,1±0,59	19,2±2,90
I experimental	21,4±0,77	14,1±0,48	18,8±4,08
II experimental	21,2±0,69	13,7±0,96	18,7±3,89
III experimental	19,9±0,59	12,9±0,59	20,7±3,03

Note: The degree of probability for the control group: * - p <0,05.

The data presented in Table 4 demonstrate that the introduction of the experimental substance positively influenced the factors of non-specific protection, particularly, it caused increased phagocytic activity of neutrophils and phagocytic index alongside with the background of a slight decrease in the quantity of circulating immune complexes. It was found that the use of Bivir-P during 14 days caused an increase in the level of Phagocytic activity neutrophils for animals of the 1st and 2nd experimental groups, where this figure was 21.7 and 21.8% (p <0.05), the same tendency persisted on the 28th day of the experiment. In addition, it was found out that in the third experimental group of animals the level of phagocytic activity neutrophils decreased contrarily on the 28th day.

After determining the phagocyte index on the 14th day of the experiment, it was figured out that in the 1st and 2nd experimental groups of animals it increased by 8.3% and 6.6%, respectively as compared to the control group. On day 28 of the experiment phagocytic index in the 1st and 2nd experimental groups was higher by 7.9 and 6.3% respectively and for the animals of the third experimental group this indicator decreased compared with the values of the 1st and 2nd experimental groups. In rehabilitation period, in the 1st and 2nd experimental groups of animals, FI slightly decreased in comparison with the 28th day of the experiment, but was still higher by 7.6 and 4.6% respectively than in the control group.

While evaluating the quantity of circulating immune complexes in the 1st and 2nd experimental groups of animals for the whole term of the experiment, a slight decrease of it was observed. However, it is worth mentioning that the animals of the 3rd experimental group demonstrated an increase of this indicator by 15.6%.

CONCLUSION

1. The use of Biovir-P at a dose of 12.5 mg/kg body weight under the condition of a subacute experiment caused an increase in the concentration of hemoglobin, the number of leukocytes, erythrocytes, γ-globulins, and phagocytic activity neutrophils by 8.6, 25.5, 7 , 16.7 and 10.9% respectively in comparison with the control group values.
2. The long-term use of Bovir-P at a dose of 125 mg/kg body weight tends to decrease the hemoglobin concentration, the absolute content of erythrocytes, leukocytes, γ-globulins and phagocytic activity neutrophils by 9.5, 6.2, 9.8, 2.2 and 0.5%, respectively compared to the control group values.

Prospects for further research: Study of the effectiveness of the tested product for the influence of xenobiotics on the organism of farm animals.

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