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[1*]G.A. Tussupbekova, [1]S.T. Tuleukhanov, [1]N.T. Ablaikhanova, [2]Yu.A. Kim,
[1]Zh.T. Abdrassulova, [1]M.S. Kulbaeva, [1]A.I. Zhussupova, [1]A. Ydyrys

[1]Laboratory of Chronobiology and Ecological Physiology, Almaty, Kazakhstan
[2]Institute of Cell Biophysics Russian Academy of Sciences, Pushchino, Moskovskaya obl., Russia
*e-mail:gulmira.ablaikizi@gmail.com

Study of the chronic toxicity of the "Virospan" drug

Abstract: The living conditions of certain groups of Kazakhstani population, especially in regions and rural areas, where adverse factors (both environmental and anthropogenic), i.e. the quality of atmospheric air, food, and drinking water are deteriorating, might negatively affect the immune status of inhabitants and lead to the occurrence of diseases. For that reason, the restoration of immunological disorders is an urgent task, since the majority of chronic, somatic, infectious diseases are accompanied by secondary immunological failure. The algorithm of immunomodulation involves the use of pharmacological agents that can increase (immunostimulation) or reduce (immunosuppression) the level of immune response. The chronic toxicity of the immunostimulating "Virospan" drug was studied in doses of 39 mg/kg, 13 mg/kg, 4.3 mg/kg. Intranasal administration of the "Virospan" drug at a dose of 4.5 and 13 mg/kg causes a statistically significant ($P \leq 0.001$) increase in the level of white blood cells, erythrocytes, hemoglobin and hematocrit. Against the background of a statistically significant ($P \leq 0.001$) decrease in the level of polymorphonuclear neutrophils, an increase in lymphocytes was observed. "Virospan" at a concentration of 39 mg/kg leads to a decrease in the total number of leukocytes by 60 days and does not cause pronounced changes in the indicators of red blood, eosinophils and basophils.
Key words: immunostimulatory drug, chronic toxicity, hematological indicators, erythrocyte, leukocyte, body mass.

Introduction

The living conditions of certain groups of Kazakhstani population, especially in regions and rural areas, where adverse factors (both environmental and anthropogenic), i.e. the quality of atmospheric air, food, and drinking water are deteriorating, might negatively affect the immune status of inhabitants and lead to the occurrence of diseases [1-3]. For that reason, the restoration of immunological disorders is an urgent task, since the majority of chronic, somatic, infectious diseases are accompanied by secondary immunological failure [4; 5]. The algorithm of immunomodulation involves the use of pharmacological agents that can increase (immunostimulation) or reduce (immunosuppression) the level of immune response [6-9].

Therapy with drugs of natural origin is widely used in global medical practice. Herbal preparations are used for the treatment and prevention, as well as in the complex therapy of various diseases [10-13]. Often, they are used without a doctor's prescription, while patients are at risk of exceeding the prescribed therapeutic dose until the appearance of side effects [14; 15]. Therefore, the study of the toxicity of the medicinal product of plant origin is necessary at the stage of preclinical evaluation.

Herbal preparation is a combination of different groups of biologically active compounds. Due to the complexity of herbal remedies, it is difficult to identify the component that causes its pharmacological activity. Search and study of biologically active compounds, organic drugs of natural origin are important for understanding its mechanism of action.

The aim of our work was to study the chronic toxicity of the plant-derived immunostimulating drug "Virospan".

Materials and methods

Intra-nasal preparations were administered to rats of the experimental groups: "Virospan" in doses of 39 mg/kg, 13 mg/kg, 4.3 mg/kg, once in 5 days [6]. After the procedure, the visual observation of experi-

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mental animals was carried out after 2, 4, 6 hours. The results on certain groups of rats were scored at 6, 30 and 60 days after the first injection of the substance and peripheral blood was taken.

Throughout the experiment, the animals were monitored daily: food and water consumption, condition of the hairline, mucous membranes and general condition (body weight dynamics, rectal temperature) were noted. General condition was assessed by daily inspection of animals. Weighing, measuring rectal temperature, water and feed consumption were performed once a week.

The blood of experimental animals was collected in a vacutainer with K3EDTA, mixed 10 times to eliminate the formation of microbunches and delivered to the laboratory. To assess hematological parameters, the Complete blood count was used to perform a complete blood count using the Siemens ADVIA 2120 automatic hematology analyzer (Germany) in the CBC/5-DIFF mode.

The following indicators were used: WBC – leukocytes (absolute number), RBC – erythrocytes (absolute number), HGB – hemoglobin (concentration), HCT – hematocrit (percentage), MCV – average red blood cell count, MCH – average hemoglobin content in a single erythrocyte, MCHC – average hemoglobin concentration in the erythrocyte mass, RDW – red blood cell distribution width, PLT – platelets (absolute), MPV – average platelet volume, NEUTRO% – neutrophils (relative col.), NEUTRO abs – neutrophils (absolute col.), LYMPHO% – lymphocytes (relative col.), Lympho abs – lymphocyte (absolute col.), MONO% – monocytes (relative col.), MONO abs – monocytes (absolute col.), BASO% – basophiles (relative col.), BASO abs – basophiles (absolute col.), EOS% – eosinophils (relative col.), EOS abs – eosinophils (absolute col.)

Statistical data processing was performed with the determination of the mean, standard and standard deviation, statistical error of the average and the percentage of differences. When determining the reliability of the difference between the indicators of the compared groups, the t-criterion of reliability was calculated, the value of P was determined from the table of Student values, the changes were considered significant at $P \leq 0.001$. All data were calculated in the MS Office Excel software package.

Results and discussion

To study the toxicity of the drug "Virospan" at a concentration of 4.3; 13; hemocytograms of experimental animals were analyzed for 39 mg/kg (Table 1).

As can be seen from the results of the Table 1, the following changes in hematological indices were observed on the background of the dosage of "Virospan" 4.3 mg/kg in laboratory animals – the total number of leukocytes in the dynamics of the study underwent a wave-like change; times and reached $10.54 \pm 0.23 \times 10^9$/L. in comparison with the control – $2.49 \pm 0.24 \times 10^9$/L, by the 30th day it decreased by 2 times to $5.45 \pm 0.23 \times 10^9$/L and by the 30th day of the experiment it increased even more to 13.04±0.23 while all changes were statistically significant ($P \leq 0.001$). The total number of erythrocytes practically did not change, so the average value on the 6th day in comparison with the control was 7.38±0.20, on 30 – 8.57±0.20 and by the 60th day of the experimental study it was $9.61 \pm 0.20 \times 10^9$/L. The hemoglobin concentration changed insignificantly, in comparison with the control – 135.00±4.82 g/L and reached 121.12±2.34 g/L, by the end of the experiment increased it significantly up to 166.12±2.34 g/L. The hematocrit also statistically significantly increased from 46.18±1.73 % to 54.15±2.32, which indicates the thickening of the peripheral blood of experimental animals.

The erythrocyte coefficients did not change significantly throughout the whole experiment, including the average red blood cell volume, the average hemoglobin content in a single red blood cell, the average hemoglobin concentration in the red blood cell mass, the calculated width of the red blood cell distribution by volume. Despite the fact that in some cases there were statistically significant differences, the values remained within the physiological norm.

The total number of platelets significantly reduced, so in the control this indicator was $911.60 \pm 31.27 \times 10^9$/L, after 69 days of the experiment $927.80 \pm 39.82 \times 10^9$/L, and by the 30th and 60th day of the experiment it was 637.80±39.82 and $741.80 \pm 39.82 \times 10^9$/L, respectively. It should be noted that the dynamics of the study showed a statistically significant ($P \leq 0.001$) decrease in the level of neutrophils from 17.50±4.98% to 2.36±0.26%, in turn, the level of lymphocytes was statistically 0.34±0.19 % to 65.94±0.50% by the end of the experiment, which may indicate activation of the cellular component of the immune system, and some immunomodulating properties, and requires further detailed study of the mechanisms of the drug's action. Along with the change in the level of lymphocytes, throughout the experiment, wavy dynamics of monocytes was observed. In the control group, the level of monocytes

was 27.00±2.37% and up to 30 days statistically significantly increased to 44.88±0.35%, but by 60 days it decreased to 14.18±0.35%. According to the level of basophils and eosinophils, it is possible to judge the absence of allergenic properties of the drug "Virospan" 4.3 mg/kg.

Table 1 – Haematological parameters of rats on the background of the dosage of "Virospan" 4.3 mg/kg

Indicators	Control	6 days	30 days	60 days
WBC	2.49±0.24	10.54±0.23*	5.45±0.23*	13.04±0.23*
RBC	7.95±0.45	7.38±0.20	8.57±0.20	9.61±0.20*
HGB	135.00±4.82	121.12±2.34*	156.12±2.34*	166.12±2.34*
HCT	46.18±1.73	40.05±2.32*	47.75±2.32	54.15±2.32*
MCV	55.46±1.34	53.96±0.19*	55.56±0.19	56.16±0.19
MCH	16.18±0.27	16.09±0.18	17.99±0.18*	17.09±0.18*
MCHC	29.24±0.29	30.61±0.93	33.11±0.93*	31.11±0.93*
RDW	13.78±0.51	16.39±0.18*	10.69±0.18*	11.39±0.18*
PLT	911.60±31.27	927.80±39.82	637.80±39.82*	741.80±39.82*
MPV	6.98±0.08	6.59±0.18*	7.49±0.18*	6.99±0.18
NEUTRO %	17.50±4.98	5.16±0.26*	3.46±0.26*	2.36±0.26*
NEUTRO abs	0.42±0,17	0.62±0.18*	0.28±0.17	0.38±0.18
LYMPHO %	0.34±0.19	55.04±0.50*	26.34±0.50*	65.94±0.50*
LYMPHO abs	0.14±0,08	5.87±0.18*	1.53±0.18*	8.66±0.18*
MONO%	27.00±2.37	20.18±0.35*	44.88±0.35*	14.18±0.35*
MONO abs	4.36±1.22	2.29±0.35*	2.61±0.35*	1.46±0.35*
BASO %	0.20±0.09	0.49±0.18	0.29±0.18	0.23±0.12
BASO abs	0.10±0.06	0.19±0.11	0.17±0.11	0.17±0.11
EOS %	0.94±0.95	0.39±0.18	2.19±0.18*	1.59±0.18
EOS abs	0.08±0.05	0.19±0.11	0.26±0.12*	0.32±0.13*
Note: * statistically significant with respect to the control (P≤0.001)				

An analysis of Figure 1 suggests that the tendency to an increase in the level of leukocytes by 4-6 times when taking "Virospan" 13 mg/kg is preserved. So by 6 days in this concentration, the total number of leukocytes increased to 8.17±0.23, by 30 days to 10.96±0.23 and on the 60th day of the experimental study it was 12.53±0.23, in all cases the changes were statistically significant (P≤0.001).

In terms of red blood, there was a thickening, a relative increase in the level of red blood cells up to 10.46±0.20, hemoglobin – to 172.12±2.34 g/L and hematocrit to 56.95±2.32%, the cause of this phenomenon it is necessary to evaluate together with the assessment of the behavioral activity of experimental animals, for the objectification of conclusions.

As for erythrocyte coefficients, the average red blood cell volume, the average hemoglobin content in a single red blood cell, the average hemoglobin concentration in the red blood cell mass, the calculated width of the red blood cell distribution by volume changed insignificantly, and most likely due to blood clotting phenomena.

The total number of platelets statistically reliably changed in the direction of decrease and increase, despite significant differences remained within the physiological norm. In addition, when taking "Virospan" 13 mg/kg, the trend towards a statistically significant decrease in segmented neutrophils from 7.50±4.98% to 5.96±0.26 to 60 days of the experiment, at the same time, the percentage of lymphocytes was statistically maintained. significantly increased and amounted to 6 days 68.64±0.50% to 60 13.64±0.50%.

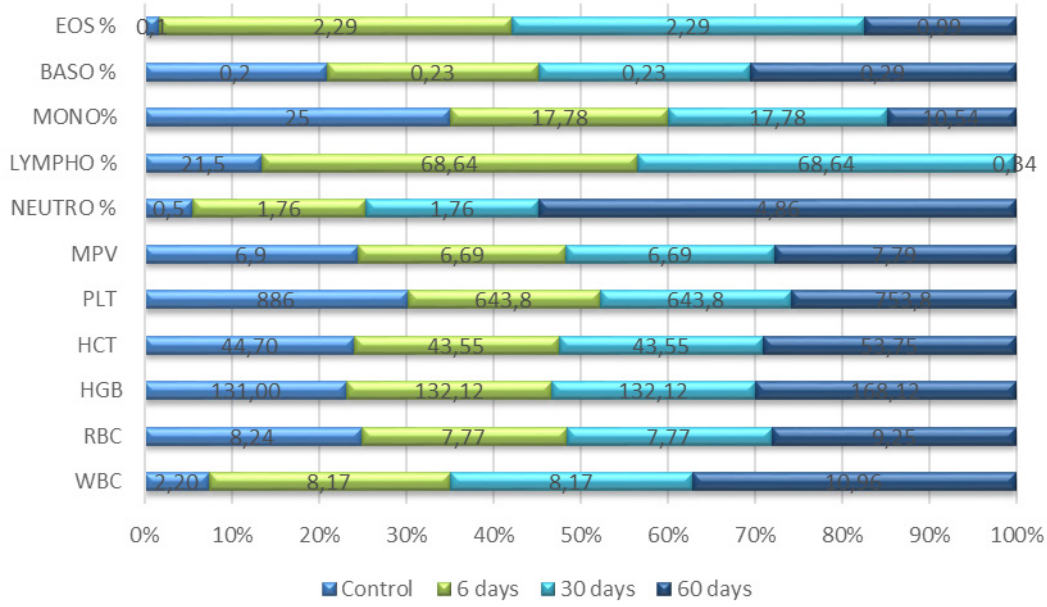

Figure 1 – Hematological parameters of rats in the background dosage of “Virospan” 13 mg/kg

Analyzing the level of monocytes in the peripheral blood of experimental animals, it should be noted that there was both a decrease and an increase in this indicator by the end of the experimental study to 77.68±0.35%. As in the case with the dosage of “Virospan” 4.5 mg/kg, “Virospan” at a dose of 13 mg/kg does not stimulate the release of histamine-producing cells into the blood and is therefore not allergenic.

The analysis of the data from the Table 2 demonstrates that the total number of leukocytes statistically significantly increases to 3.08±0.23 to 6 days, to 10.04±0.23 to 30 days and statistically significantly decreases to 60 days to 2.07±0.23, which has the opposite effect compared with a concentration of “Virospan” 4.5 and 13 mg/kg.

So, red blood indices increased, including the level of erythrocytes in dynamics reached $9.69\pm0.20\times10^{12}$/L, and the hemoglobin concentration 165.12±2.34 g/l, however, the hematocrit remained within the normal range and statistically did not change and amounted to 49.95±2.32%.

Table 2 – Hematological parameters of rats on the background of the dosage of “Virospan” 39mg/kg

Indicators	Control	6 days	30 days	60 days
WBC	2.49±0.24	3.08±0.23*	10.04±0.23*	2.07±0.3
RBC	7.95±0.45	8.13±0.20	9.34±0.20*	9.69±0.20*
HGB	135.00±4.82	141.2±2.34	161.12±2.34*	165.12±2.34*
HCT	46.18±1.73	46.35±2.32	50.35±2.32	49.95±2.32
MCV	55.46±1.34	56.76±0.19	53.66±0.19*	51.26±0.19*
MCH	16.18±0.27	17.09±0.18*	17.09±0.18*	16.89±0.18*
MCHC	29.24±0.29	30.81±0.93*	32.51±0.93*	33.51±0.93*
RDW	13.78±0.51	12.79±0.18*	12.09±0.18*	11.39±0.18*
PLT	911.60±31.27	1125.80±39.82*	891.80±39.82	639.80±39.82*
MPV	6.98±0.08	6.79±0.18	7.49±0.18*	6.09±0.18*
NEUTRO %	17.50±4.98	10.86±0.26*	4.26±0.26*	17.56±0.26*
NEUTRO abs	0.42±0.17	0.42±0.18	0.50±0.18	0.45±0.18

Continuation of table 2

Indicators	Control	6 days	30 days	60 days
LYMPHO %	0.34±0.19	0.44±0.41*	4.74±0.50*	0.44±0.41*
LYMPHO abs	0.14±0.08	0.19±0.10	0.55±0.18	0.19±0.10
MONO%	27.00±2.37	10.48±0.29*	66.28±0.35*	25.70±1.08
MONO abs	4.36±1.22	3.44±0.35	6.82±0.35*	5.69±0.35
BASO %	0.20±0.09	0.39±0.18	0.59±0.18*	0.29±0.18
BASO abs	0.10±0.06	0.17±0.11	0.20±0.11	0.17±0.11
EOS %	0.94±0.95	1.29±0.18	2.89±0.18*	4.99±0.18*
EOS abs	0.08±0.05	0.20±0.11	0.39±0.15*	0.24±0.11
Note: * statistically significant with respect to the control (P≤0.001)				

The average volume of the erythrocyte, the average hemoglobin content in a single erythrocyte, the average concentration of hemoglobin in the erythrocyte mass, the calculated width of the distribution of erythrocytes by volume did not change significantly. Red blood indicators in this case indicate a better tolerance of the drug concentration at 39 mg/kg, as well as probably the inclusion of compensatory mechanisms and the absence of dehydration of the organism of experimental animals. The total number of platelets reaches its peak by 6 days – 1125.80±39.82 and by the end of the experiment it decreases 2-fold to 639.80±39.82. In terms of white blood, it should be noted that the percentage of neutrophils in the dynamics is statistically significantly reduced by 30 days to 4.26±0.26, but by 60 days it increases again to control values – 17.56±0.26. The level of lymphocytes varies slightly and the highest value is by 30 days – 4.74±0.50%, decreasing by 60 days to 0.44±0.41%. Analyzing the level of monocytes, it should be noted that the highest value – 66.28±0.35% was also noted on the 30th day of the experimental study. As in the case with the dosage of "Virospan" 4.5 mg/kg, 13 mg/kg at a dose of 39 mg/kg, a significant increase in the level of eosinophils and basophils was not observed.

Conclusion

Thus, according to a detailed analysis of peripheral blood against the background of the dosage of the drug Virospan, it was found that under experimental conditions, at 4.5 and 13mg/kg, "Virospan" causes a statistically significant increase in the level of white blood cells, red blood cells, hemoglobin and hematocrit. Against the background of a statistically significant decrease in the level of polymorphonuclear neutrophils, an increase in the level of lymphocytes was observed. "Virospan" at a concentration of 39 mg/kg demonstrates a statistically significant decrease to 60 days of the total number of leukocytes, the absence of pronounced changes in the indicators of red blood and the absence of changes in the level of eosinophils and basophils. In general, to exclude the general toxic effect of the studied drug, a comprehensive assessment of their properties in vivo, namely, biochemical, anatomical and morphological parameters is necessary.

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