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## Screening drugs-potential immunomodulators for T-2 mycotoxicosis



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### ABSTRACT

The aim of the research was to study the effectiveness of substances with an immunostimulating effect in T-2 mycotoxicosis. Subacute T-2 mycotoxicosis was simulated in male, white Wistar rats by administering intragastrically, a toxic at a dose of 1/5 LD<sub>50</sub> (0.64 mg/kg of body weight) for 15 days, at the same time the animals were immunized with the vaccine against colibacteriosis (on the first day of the experiment). The following drugs were tested: "Xymedon" (1-(β-oxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxypyrimidine) was administered intragastrically at a dose of 75 mg/kg daily; "Dimephosphone" (Dimethylxobuthylphosphonilmethylate) at a dose of 90 mg/kg daily; "Levamisole" (S)-2,3,5,6-Tetrahydro-5-phenylimidazo[2,1-b]thiazole hydrochloride) intragastrically at a dose of 4 mg/kg for the first 3 days, after a 4-day break it was administered again for 3 days; "Thymalin" (thymus extract derived from thymus

glands of large animals) administered intramuscularly at a dose of 0.2 mg/kg for 5 days from 7th day of the experiment. All experimental animals were immunized with the vaccine against colibacteriosis; the vaccine was administered intramuscularly into the back of the thigh at a dose of 0.5 ml per day. The criteria for evaluating the effectiveness of the drugs were hematological indicators, immunological indicators and accumulation of specific antibodies to the vaccine. It has been established that all the tested drugs had a protective effect which was expressed in positive changes in hematological, immunological and non-specific resistance indicators. "Thymalin" had the most pronounced protective effect in toxicosis with T-2 toxin in rats, "Xymedon" had the least protective effect, "Dimephosphone" had an average level of effect. "Thymalin" proved to be more effective according to the indicators of the accumulation of specific antibodies.

**Keywords:** T-2 toxin, immunosuppression, immunomodulators

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### INTRODUCTION

Mycotoxins contaminate food and feed at all stages of their production, transportation, storage, processing, and marketing. The researchers' interest is in the immunosuppressive properties of mycotoxins, especially T-2 toxin.<sup>1-7</sup> We cannot exclude the possibility of the effect of several mycotoxins or combined effect of mycotoxins and other eco-toxicants.<sup>8-11</sup>

Various adsorbents<sup>11,12,13,14,15</sup> or chemical compounds with antioxidant properties<sup>16</sup> are the most widely used to protect animals from the harmful effect of mycotoxins. Taking into account the pronounced immunosuppressive effect of mycotoxin T-2, it is interesting to study the effectiveness of the application of the substances with immunostimulatory or potential immunostimulatory effect in T-2 mycotoxicosis.

### MATERIALS AND METHODS

For screening, drugs such as xymedon (1-(β-oxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxypyrimidine), dimephosphone (Dimethylxobuthyl

phosphonilmethylate), levamisole (S)-2,3,5,6-Tetrahydro-5-phenylimidazo[2,1-b]thiazole hydrochloride) and thymalin (thymus extract derived from thymus glands of large animals) were used, their direct or indirect immunostimulatory effects were established.<sup>17,18,19</sup>

The experiment was conducted in laboratory animals – white Wistar rats from the special nursery in FGBNU "FCTRB-VNIVI". Before the experiment the animals were kept in 2-week quarantine, fed according to the norms. The food was also examined on biological safety – the presence of pathogenic and opportunistic pathogenic microflora, toxins; the food corresponded to the Security Quality Certification.

The experiment was conducted in 100 adult male white Wistar rats weighing 160–180 g, they were divided into 5 groups of 20 animals in each according to the principle of analogs. T-2 toxin was administered intragastrically to the animals of the first group in the form of a 5% aqueous alcoholic solution at a dose of 1/5 LD<sub>50</sub> (0.64 mg/kg of body weight) for 15 days daily. The rats of the second group received intragastrically T-2 toxin in the form of a 5% aqueous alcoholic solution at a dose of

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1/5 LD<sub>50</sub> daily and immunostimulant “Levamisole” at a dose of 4 mg/kg for the first 3 days, after a 4-day break they received it again for 3 days. T-2 toxin (1/5 LD<sub>50</sub>) and “Xymedon” at a dose of 75 mg/kg were administered to the animals of the third group daily. T-2 toxin and “Dimephosphone” at a dose of 90 mg/kg were administered to the rats of the fourth group daily. Animals of the fifth group received intragastrically T-2 toxin (1/5 LD<sub>50</sub>) daily and “Thymalin” intramuscularly at a dose of 0.2 mg/kg for the first 5 days. The experiment lasted 15 days. All the animals were immunized with the vaccine against colibacteriosis, prepared in FGBNU “FCTRB-VNIVI” (Kazan, Russia), kindly provided by the head of the young animal disease laboratory. The vaccine was administered intramuscularly into the back of the thigh at a dose of 0.5 ml per day. To carry out the experiment, crystalline T-2 toxin (Fermentek Ltd, Israel) was used, the purity of the toxin was not less than 97%.

Such indicators as clinical features, hematological indicators, indicators of nonspecific resistance of rats, T- and B- lymphocyte content and accumulation of specific antibodies to the vaccine against colibacillosis served as control. The obtained data were compared to the background.

Blood samples were taken from the experimental and control animals by decapitation. Hematological studies – the determination of the number of erythrocytes, leukocytes, and hemoglobin were carried out with the Mythic 18 hematology analyzer. Titers of antibodies to the vaccine against colibacteriosis in piglets were determined by serum agglutination test.

The activity of lysozyme in serum was examined by the method based on the ability of lysozyme to dissolve the mucopolysaccharides of the shell of certain bacteria. One-day culture *Micrococcus lysodeikticus* served as a standard to determine the titer of lysozyme in the test material.

The level of T- lymphocytes in peripheral blood was determined by the spontaneous rosette test with heterogeneous red blood cells (E-rosetting). The method is based on the ability of thymus-dependent lymphocytes to form spontaneous rosettes with red blood cells due to the presence of T- lymphocytes on the membrane that form an immune relationship with the surface antigens of heterologous erythrocytes.

The identification of B- lymphocytes was carried out by EAC-rosetting. The principle of the method lies in the interaction of the membrane of B-cells that contains receptors for the third component of complement (C3) with red blood cells “loaded” with a complement. As a result, rosettes are formed.

The phagocytic ability of neutrophils was determined by phagocytosis indicators: phagocytic activity – the percentage of active (phagocytic) neutrophils; phagocytic index – the average number of microbial bodies per counted neutrophil; phagocytic number – the average number of microbes in one active neutrophil, phagocytic capacity that characterizes the total phagocytic activity and depends on the number of leukocytes in 1mm.<sup>3</sup> The object of phagocytosis was a one-day culture of *Staphylococcus aureus*.

Numerical data processing was performed with the method of statistical variance with the use of Student’s validation criterion.

## RESULTS AND DISCUSSION

The dynamics of changes in hematological indicators in the case of T-2 toxicosis when applying immunostimulants is presented in Table 1.

The data presented in Table 1 show that in the animals in all the groups there was a decrease in the number of erythrocytes, leukocytes, and hemoglobin. Thus, on 7th and 15th days in the first group of the animals that received the toxin without any immunostimulants (“toxicity control”), the number of erythrocytes reduced by 7.2% and 24.3%; leukocytes – by 7.3% and 26.4%; the content of hemoglobin - by 1.8% and 11.6%, respectively.

In the second group of animals where levamisole was administered there was a slight decrease in the number of erythrocytes, leukocytes and hemoglobin - 3.7%; 3.6% and 0.7% on 7th day; however, on

**Table 1** Hematological indicators in white rats in experimental T-2 toxicosis when applying immunostimulants (n=10)

| The day of the research | Group | Indicator                          |                                 |                 |
|-------------------------|-------|------------------------------------|---------------------------------|-----------------|
|                         |       | erythrocytes, x10 <sup>12</sup> /l | leukocytes, x10 <sup>9</sup> /l | hemoglobin, g/l |
| Background              |       | 7.34±0.23                          | 9.52±0.41                       | 152.50±4.0      |
| 7                       | 1     | 6.81±0.21                          | 8.83±0.21                       | 149.80±3.9      |
|                         | 2     | 7.07±0.25                          | 9.18±0.19                       | 151.40±4.1      |
|                         | 3     | 7.06±0.22                          | 9.18±0.29                       | 150.10±3.8      |
|                         | 4     | 7.04±0.21                          | 9.23±0.24                       | 150.20±3.7      |
|                         | 5     | 7.05±0.29                          | 9.21±0.22                       | 150.10±3.8      |
| 15                      | 1     | 5.56±0.19***                       | 7.01±0.23***                    | 144.80±3.5      |
|                         | 2     | 5.88±0.27*                         | 7.48±0.21***                    | 146.20±3.7      |
|                         | 3     | 5.97±0.24*                         | 7.37±0.27***                    | 145.10±2.9      |
|                         | 4     | 6.18±0.26                          | 7.95±0.24**                     | 147.20±3.4      |
|                         | 5     | 6.14±0.27                          | 8.07±0.28*                      | 147.50±4.6      |

\* – p < 0.05; \*\* – p < 0.01; \*\*\* – p < 0.001

**Table 2** Indicators of nonspecific resistance of white rats in experimental T-2 toxicosis when applying immunostimulants (n=10)

| The day of the research | Group | Indicator                 |           |                          | Activity of lysozyme, % |
|-------------------------|-------|---------------------------|-----------|--------------------------|-------------------------|
|                         |       | PA, %                     | PN, unit  | PC, 10 <sup>9</sup> /l   |                         |
| Background              |       | 63.93±1.17                | 5.22±0.47 | 49.69±3.04               | 46.25±0.81              |
| 7                       | 1     | 66.82±1.34                | 5.40±0.39 | 47.68±3.17               | 44.74±1.07              |
|                         | 2     | 67.71±1.57                | 5.36±0.51 | 49.20±2.57               | 45.23±0.93              |
|                         | 3     | 65.73±1.19                | 5.38±0.49 | 49.39±3.08               | 45.07±1.17              |
|                         | 4     | 64.94±1.07                | 5.41±0.62 | 49.93±2.75               | 45.14±1.09              |
|                         | 5     | 65.82±1.12                | 5.41±0.54 | 49.83±3.19               | 44.93±0.87              |
| 15                      | 1     | 54.87±1.28 <sup>***</sup> | 4.60±0.56 | 32.25±3.41 <sup>**</sup> | 42.52±0.96 <sup>*</sup> |
|                         | 2     | 56.36±1.39 <sup>**</sup>  | 4.83±0.74 | 36.13±3.82 <sup>*</sup>  | 42.53±1.24 <sup>*</sup> |
|                         | 3     | 58.05±1.57 <sup>*</sup>   | 4.87±0.68 | 35.89±2.94 <sup>**</sup> | 42.96±0.98 <sup>*</sup> |
|                         | 4     | 59.94±1.49                | 5.26±0.59 | 41.82±3.79               | 42.71±1.29 <sup>*</sup> |
|                         | 5     | 59.06±1.61 <sup>*</sup>   | 5.03±0.73 | 40.59±3.19               | 42.82±1.34              |

<sup>\*</sup> - p < 0.05; <sup>\*\*</sup> - p < 0.01; <sup>\*\*\*</sup> - p < 0.001

**Table 3** The content of T- and B- lymphocytes in the blood of white rats in experimental T-2 toxicosis when applying immunostimulants (n=10)

| The day of the research | Group | Indicator                  |                           |
|-------------------------|-------|----------------------------|---------------------------|
|                         |       | T- lymphocytes, %          | B- lymphocytes, %         |
| Background              |       | 55.34±0.78                 | 22.54±0.51                |
|                         | 1     | 46.37±0.57 <sup>***</sup>  | 20.06± 0.59 <sup>**</sup> |
|                         | 2     | 48.32± 1.18 <sup>***</sup> | 20.53± 0.53 <sup>*</sup>  |
| 15                      | 3     | 48.84± 1.12 <sup>***</sup> | 20.68± 0.69               |
|                         | 4     | 50.08± 0.59 <sup>***</sup> | 21.17± 0.68               |
|                         | 5     | 50.84± 0.73 <sup>**</sup>  | 21.13± 0.71               |

<sup>\*</sup> - p ≤ 0.05; <sup>\*\*</sup> - p ≤ 0.01; <sup>\*\*\*</sup> - p ≤ 0.001

**Table 4** The content of antibodies in the serum agglutination in rats after vaccination when applying immunostimulants in subacute T-2 toxicosis (n=6)

| The dilution of serum | The group of animals |   |   |   |   |
|-----------------------|----------------------|---|---|---|---|
|                       | 1                    | 2 | 3 | 4 | 5 |
| 1:5                   | +                    | + | + | + | + |
| 1:10                  | +                    | + | + | + | + |
| 1:20                  | +                    | + | + | + | + |
| 1:40                  | +                    | + | + | + | + |
| 1:80                  | -                    | + | + | + | + |
| 1:160                 | -                    | - | + | + | + |
| 1:320                 | -                    | - | + | - | + |
| 1:640                 | -                    | - | - | - | - |
| 1:1280                | -                    | - | - | - | - |
| Control               | -                    | - | - | - | - |

15th day a decrease was more significant - 19,9%; 21.4% and 9.4%, respectively, in comparison with the background, but less pronounced than in the first group of rats.

In the third group of animals where xymedon was administered the content of erythrocytes, leukocytes and hemoglobin did not significantly differ from that in the second group and decreased by 3.8%; 3.6% and 1.6% on 7th day; on 15th day xymedon had significant protective effect on hematological indicators which decreased by 18.7%; 22.6% and 10.1%, respectively, in comparison with the background. But this reduction was less pronounced than in the toxicity control group. In the fourth group of animals where dimephosphone was administered there was a decrease of 4.1%, 3.1% and 1.6% in the number of erythrocytes, leukocytes, and hemoglobin on the 7th day; on 15th day - 15.8%; 16.5% and 8.7%, respectively. In the fifth group of animals where thymalin was administered there was a decrease of 4.0%; 3.3% and 1.6% in the number of erythrocytes, leukocytes and hemoglobin on the 7th day; on 15th day - 16.4%; 15.2% and 9.2%, respectively.

The results of the experiment show that the application of immunostimulants "Xymedon", "Dimephosphone" and "Thymalin" had a protective effect on hematological indicators in animals.

The results of the research on nonspecific resistance of rats after administering T-2 mycotoxin in combination with immunostimulants are presented in Table 2.

The data presented in Table 2 show that the activity of lysozyme in the animals in the first group decreased by 3.3% and 8.1% on 7th and 15th days; in the second group - by 2.2% and 8.0%; in the third group - by 2.6% and 7.1%, in the fourth group - by 2.4% and 7.7%, in the fifth group - by 2.9% and 7.4%, respectively.

The phagocytic activity in rats in the first group increased by 4.5% on the 7th day and decreased by 14.2% on the 15th day. In the second group, it increased by 5.9% on the 7th day and decreased by 11.8% on the 15th day. In the third group, it increased by 2.8% on the 7th day and decreased by 9.2% on the 15th day. In the fourth group of animals, the phagocytic activity in rats increased by 1.6% on the 7th day and decreased by 6.2% on the 15th day. In the fifth group, it increased by 3.0% on the 7th day and decreased by 7.6% on the 15th day. The phagocytic number in the first group of animals increased by 3.5% on the 7th day and decreased by 11.9% on the 15th day. In the second group, there was an increase of 2.7% in phagocytic number on the 7th day and a decrease of 7.5%

on the 15th day. In the third group of animals, it increased by 3.1% on the 7th day and decreased by 6.7% on the 15th day. In the fourth group, the phagocytic number increased by 3.6% on the 7th day and increased by 0.8% on the 15th day. In the fifth group, it increased by 3.6% on the 7th day and decreased by 3.6% on the 15th day.

The phagocytic capacity in the animals of the first group decreased by 2.1% and 32.6% on the 7th day and on the 15th day, respectively. In the second group, it increased by 0.5% on the 7th day and decreased by 25.4% on the 15th day. In the third group, the phagocytic capacity decreased by 1.6% and 22.6% on the 7th day and on the 15th day, respectively. In the fourth group of animals, it decreased by 1.1% and 14.9%, respectively. In the fifth group, the phagocytic capacity decreased by 1.6% on the 7th day and 22.6% on the 15th day.

The results of studying the content of T- and B- lymphocytes on the 15th day of the experiment are shown in [Table 3](#).

The content of T- and B- lymphocytes in the blood of the animals in all the groups decreased on the 15th day. The changes were as follows: in the first group of animals (T-2 toxin at a dose of 1/5 LD<sub>50</sub> was administered), the reduction in the number of T- and B- lymphocytes was 16.2% and 11.0%, respectively. In the second group of animals (T-2 toxin and levamisole were administered) the decrease was less significant - 12.7% and 8.9%, respectively. In the third group of animals (T-2 toxin and xymedon were administered) there was a slight decrease of 11.8% and 8.3% in T- and B- cells, respectively. In the fourth group of experimental animals (T-2 toxin and dimephosphone were administered) a reduction in T- and B - lymphocytes was 9.5% and 6.1%, respectively. In the fifth group of animals (T-2 toxin and thymalin were administered) there was a minimal decrease of 8.1% and 6.3% in the number of T- and B- lymphocytes, respectively, in comparison with the background.

The results of studying the accumulation (titer) of specific antibodies in the serum of white rats at various dilutions when applying immunostimulants are presented in [Table 4](#).

The results of the research have shown that the maximum level of antibodies in the serum (titer) was observed in the third group (T-2 toxin + xymedon) and in the fifth group (T-2 toxin + thymalin) (1:320). In the first group (T-2 toxin) there was the lowest level of the titer (1:40). The titer level in the second group (T-2 toxin + levamisole) was 1:80 and in the fourth group (T-2 toxin + thymalin) - 1:160.

## CONCLUSION

The results of the experiment show that all the immunostimulants had a protective effect which was expressed in positive changes in hematological, immunological and non-specific resistance indicators. It has been established that thymalin had the most pronounced protective effect in toxicosis with the T-2 toxin in rats, xymedon had the least protective effect, and dimephosphone had an average level of effect. Thymalin proved to be more effective according to the indicators of the accumulation of specific antibodies.

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