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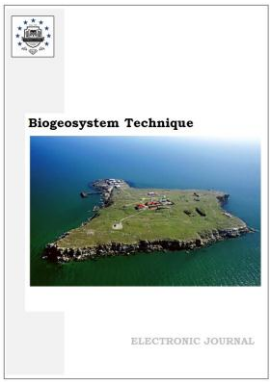

Study of Reducing the Danger of T-2 Toxin When Using a Drug of Organomineral Origin

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Abstract

The prevalence of microscopic fungi and their metabolites in food raw materials and agricultural products poses a great threat to the population. Many mycotoxins are highly toxic and resistant to various environmental factors. The drugs available on the market do not completely neutralize these toxins. The residues of toxins can pose a threat to farm animals, causing a violation in hematological, biochemical, reproductive indicators, a decrease in weight gain, a deterioration in the sanitary quality of meat, milk and eggs. The comparative studies of the T-2 toxin effect on the biological objects – protozoa and primary cell culture (*Stylonychia mytilus* and bull spermatozoa) indicate a selective toxic activity of this type mycotoxin. When protozoa exposed to T-2 toxin in a dose of 0.5 mcg/ml, cell death was 19 %. The corresponding figure for a germ cell culture was 34 %. The protective effect of exposure to T-2 toxin on biological models was observed with the use of all studied protective drugs. When using bentonite from the Apastovo deposit of RT, the decrease in the death of protozoa against the background of the influence of T-2 toxin was 15 % higher compared to the control. When using strains of microorganisms *B. Subtilis* and *L. Plantarum* together with T-2 toxin, the death of protozoa was 10 % higher than the control. The use of the drug KMBI-3 containing bentonite and strains of microorganisms B. Subtilis and L. Plantarum significantly reduced the toxic effects of T-2 toxin, both on protozoan cells and on primary germ cells. The drug KMBI-3 is a dry powder that has the potential to reduce phytopathogen toxins in food raw materials.

Keywords: biological toxins, food raw materials, feed, toxicity, protozoa, primary germ cells, liver cell culture, mycotoxins, T-2 toxin.

1. Introduction

Mycotoxins are one of the highly toxic pollutants of feed and food raw materials due to the widespread distribution of their producers – microscopic fungi in the environment. Basically, fusariotoxin – T-2 toxin is of the greatest sanitary importance in terms of frequency and prevalence in our country. T-2 toxin is a highly toxic trichothecene produced by microscopic fungi of the genus

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Fusarium spp. These microscopic fungi are present in corn, barley, wheat, oats and other cereals, which are common components of feed and food raw materials (Escrivá et al., 2015; Zgadzay et al., 2021; Dzhavakhiya et al., 2022; Miftakhov et al., 2022; Kosolapov et al., 2023). According to the frequency of occurrence, T-2 toxin is found in more than 30 % of food raw materials at a level from 10 to 735 micrograms/kg, which poses a potential threat to animals and humans (Wang et al., 2013; Kononenko et al., 2019; Bikmullin et al., 2023). The ingestion of T-2 toxin into the animal body can cause acute or chronic effects with serious disorders in feed intake, growth and development, negatively affects reproductive ability and health (Miedaner et al., 2017; Karmanov et al., 2020; Gagkaeva et al., 2023). Low concentrations of T-2 toxin in birds cause a decrease in body weight gain, egg production, egg quality and lower hatchability (Kononenko et al., 2019; Dazuk et al., 2020). The toxic effects of the T-2 toxin include inhibition of protein, DNA and RNA synthesis and the production of immunoglobulin. In addition, the T-2 toxin accelerates the production of reactive oxygen species (ROS), which causes oxidative stress, further leads to inflammatory reactions and apoptosis (Zhang et al., 2021; Sun et al., 2022). The rate of liver apoptosis and pathology in organs worsened with an increase in the concentration of T-2 toxin, which ranged from 0.5 to 2.0 mg/kg, which induced mitochondrial-mediated apoptosis by producing ROS and stimulating cytochrome translocation and apoptosome formation (Yin et al., 2020). Dose 4.0 mg/kg of T-2 toxin has been reported to cause oxidative stress and inflammatory reactions and damage to kidney function in mice (Valiullin et al., 2020; Huang et al., 2021). In addition, T-2 toxin at a dose of 0.5 mg/kg disrupted various endogenous metabolic processes, causing the accumulation of amino acids and nucleotides in the liver, kidneys and spleen (Wan et al., 2016). T-2 toxin affects tissues in a state of active and rapid division through several toxic mechanisms that cause serious damage, such as the intestines, liver, kidneys, spleen and bones (Sokolovic et al., 2008). Thus, since the T-2 toxin is widely present in animal feed, it often causes a decrease in animal productivity and tissue damage. In animals and humans, T-2 toxin can affect the hematopoietic and nervous systems, suppress humoral and cellular immunity. It has mutagenic, carcinogenic and embryotoxic effects (Lucioli et al., 2013). T-2 toxin can cause apoptosis, programmed cell death in the liver and nervous tissue (Kolf-Clauw et al., 2013).

The purpose of our research was a comparative study of the protective properties of the drug KMBI-3 when exposed to T-2 toxin on protozoa and mammalian cell cultures.

2. Methods

To study the toxic properties of the studied doses of T-2 toxin, a method for determining in vitro toxicity on protozoa was used. The research was carried out on the simplest Stylonychia mytilus (GOST 31674-2012). The infusoria were divided into five equal groups: 1) the control group without the addition of toxin and drugs; 2) the second group received T-2 toxin at a dose of 0.5 mcg/ml; 3) the third group received T–2 toxin at a dose of 0.5 mcg/ml and bentonite from the Apastovsky deposit of RT; 4) the fourth group – T-2 toxin at a dose of 0.5 mcg/ml and B. Subtilis and L. Plantarum strains; 5) the fifth group received T-2 toxin at a dose of 0.5 mcg/ml, B. Subtilis and L. Plantarum strains and bentonite from the Apastovsky deposit of RT. The assessment of cell death in infusoria was carried out on the basis of a visual analysis of their mobility. The method is based on the fact that living infusoria are in constant motion.

The cytotoxic effect of T-2 toxin on mammalian cells was evaluated according to GOST R ISO 10993-5-2009 («Express method of toxicity assessment using bovine sperm as a test object») https://internet-law.ru/gosts/gost/49188/. 3 groups were formed to study the cytotoxicity of T-2 toxin. The first group served as a control; the second group received T-2 toxin mixed with strains of B. Subtilis and L. Plantarum and bentonite from the Apastovsky deposit of RT in concentrations of 0.5 mcg/ml; the third group received T-2 toxin in concentrations of 0.5 mcg/ml.

3. Results

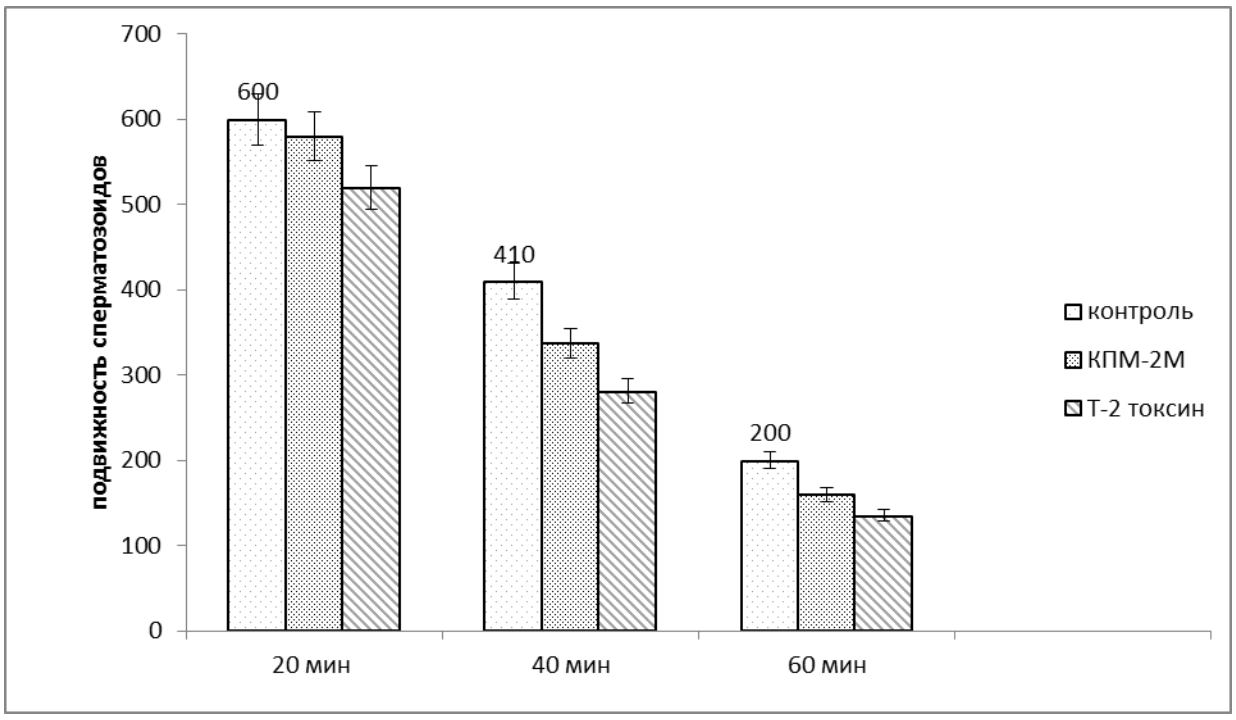
The results of studies on reducing the toxicity of T-2 toxin in protozoa are presented in the Table 1, which shows the results of studies on reducing the toxicity of T-2 toxin in protozoa.

Table 1. T-2 toxicity in protozoa

Groups	Time, %		
	20 min	40 min	60 min
1	100,0±6,5	100,0±6,5	100,0±6,1
2	95,1±6,8	90,4±6,9	81,2±5,8*
3	100,0±6,2	95,2±6,3	85,1±5,9
4	100,0±6,9	98,3±6,5	89,0±6,3
5	100,0±6,1	98,1±6,0	96,1±6,7

Reliability * p <0,05

The results of studies on the reduction of cytotoxic properties of T-2 toxin on bull spermatozoa with KMBI-3 are shown in the Figure 1.



Reliability: p <0,05

Fig. 1. Cytotoxic properties of T-2 toxin on bull spermatozoa in doses of 0.5 mkg/ml.

The viability of a cell culture under the influence of T-2 toxin against the background of the use of the protective composition KMBI-3 is shown in the Figure 2.

4. Discussion

Research on protozoa is of great interest due to the high ability to repeat the multiplicity of the experiment, as well as from the point of view of ethical standards in relation to animal research.

From the data presented in the Table 1 it can be seen that at the 20th minute of exposure to T-2 toxin on protozoa, no significant toxic properties were observed when compared with the control group, after 40th minutes in the second group, when exposed to T-2 toxin, the death of protozoa was 20 % greater compared to the control. In the third group, at the 40th minute of exposure, the death of protozoa was observed by 5 % more than in the control group. By this time, in the fourth and fifth groups, there was a slight (2 %) death of protozoa compared to the control group. By the 60th minute of exposure of T-2 toxin to infusoria in the second group, the death of protozoa was 20 %, in the third and fourth groups 15 and 10 %, respectively. In the fifth group, when using a protective drug, the negative effect of T-2 toxin was slightly lower than the control by 5 %.

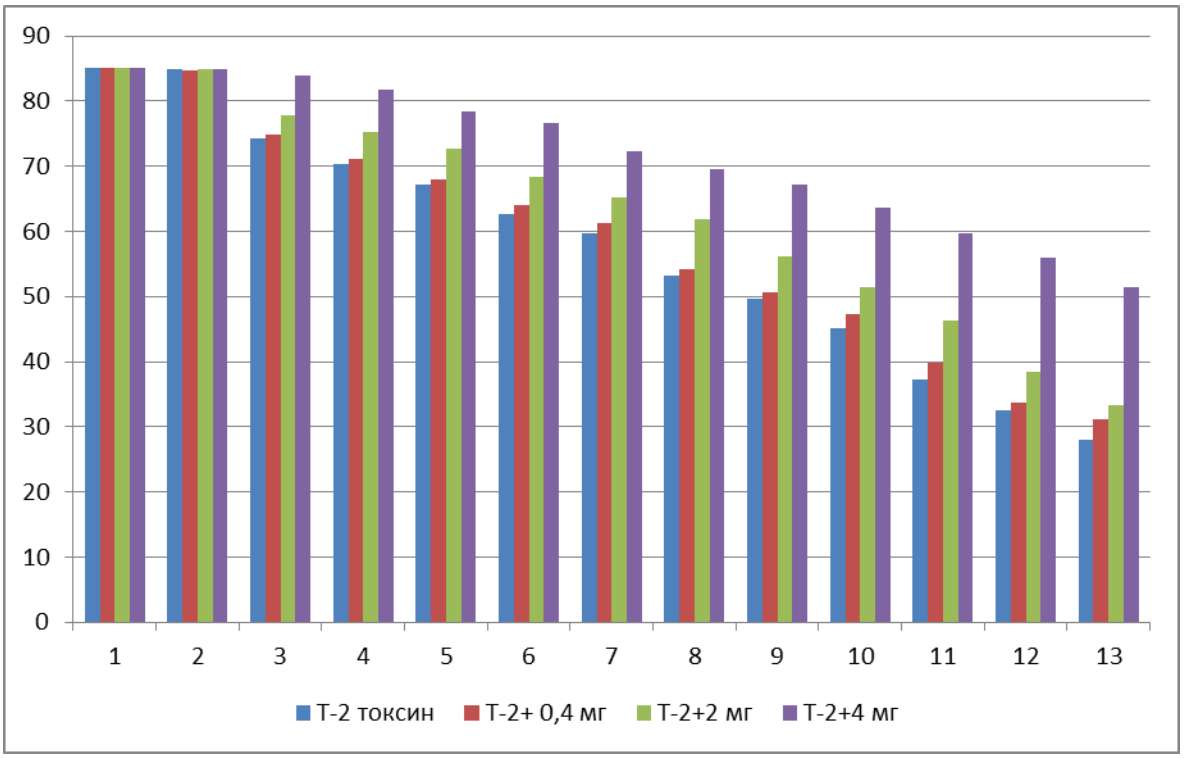


Fig. 2. Cell culture viability under the T-2 toxin against the protective composition KMBI-3 background

It can be seen from the Figure 1 that in the second group, by the 20th minute of the studies, no significant changes were observed when compared with the control group. In the third group, there was a decrease in the average sperm motility by 28 % compared to the control group.

By the 40th minute of the study, a significant change in the motility of the sperm suspension was observed in the second and third groups by 12 and 27 % lower than in the control group.

In the second group, by the 60th minute, the decrease in sperm motility was 20 % compared to the control group. In the third group, the activity of sperm suspension was 34 % lower than in the control group.

In the second series of experiments, primary liver cell culture was used to study the biological properties of T-2 toxin. The cells were cultured in DMEM medium in the presence of 10 % fetal calf serum at 37°C and 5 % CO_2. T-2 toxin was dissolved in a mixture of DMSO and 96 % alcohol in a ratio (1:1).

13 groups participated in the experiment: the first group served as a control with the addition of a mixture of DMSO and 96 % alcohol in a ratio (1:1) and without the addition of T-2 toxin; the second group received 1.07×10^{-9} T-2 toxin; the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, the eleventh, twelfth and thirteenth groups received in addition to the cells 10.7×10^{-9}, 21.5×10^{-9}, 42.9×10^{-9}, 6.4×10^{-8}, 8.6×10^{-8}, 10.7×10^{-8}, 12.9×10^{-8}, 1.5×10^{-7}, 1.7×10^{-7}, 1.9×10^{-7}, $2{,}14\times10^{-7}$ M T-2 toxins, respectively. T-2 toxin and bacterial-based compositions KMBI-3 were mixed and aged together for 6 hours and after exposure were added to a medium with a cellular monolayer. The concentration of bacterial-based compositions KMBI-3 was used for research in three variants: 0.4, 2.4 mg/ml.

Figure 2 shows that when T-2 toxin was exposed to cell culture, viability in the second, third, fourth and fifth groups decreased slightly. In the sixth, seventh, eighth, ninth, tenth, eleventh, twelfth and thirteenth groups, cell viability decreased by 26,5; 30,1; 37,5; 41,8; 47,1; 56,3; 61,9 and 66.0 % compared to the control.

When using a protective composition at a dose of 4 mg/ml, the least negative effect of T-2 toxin on cell culture was observed. Viability in the second, third, fourth, fifth, sixth and seventh groups decreased slightly. In the eighth, ninth, tenth, eleventh, twelfth and thirteenth groups, cell viability decreased relative to the control by 18.4; 21.2; 25.4; 30.1; 36.0 and 40.0 %, respectively.

5. Conclusion

The comparative studies of the T-2 toxin effect in doses of 0.5 mcg/ml on the biological objects – protozoa and primary cell culture (Stylonychia mytilus and bull spermatozoa) indicate a selective toxic activity of this type mycotoxin. When the protozoa exposed to the T-2 toxin in doses of 0.5 mcg/ml for 60 minutes, the toxicity was 18 %, and the spermatozoa exposed to the T-2 toxin at the same dose and time, their activity decreased by 34 %, respectively, compared with the control group. When using the protective composition KMBI-3 against the T-2 toxin on protozoa for 60 minutes, their survival rate was 19 % higher compared to the group without the protective drugs. When studying the possibility of the negative effects of T-2 toxin on living systems reducing, it was seen that the composition of KMBI-3, consisting of strains of *B. Subtilis* and *L. Plantarum* and bentonite from the Apastovo deposit of the Republic of Tatarstan most effectively exhibit protective properties and are of interest for further work on the biodegradation of toxins of biological origin.

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