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# A Complex Approach to Liver Protection from Mycotoxicoses in Experimental Conditions

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## Abstract

**Aim and Objective:** The main objective of this study was the research of the protective effect of a complex biologically active additive containing dietary fiber and lecithin on the model of associative mycotoxicosis reproduced on laboratory animals. **Materials and Methods:** Experimental modeling of chronic associative mycotoxicosis was performed on 30 male rats, divided into three groups of 10 individuals each. The essence of the method for the reproduction of associative mycotoxicosis was that for 30 days, the rats of the first and second experimental groups were fed with the feed that was naturally contaminated with mycotoxins. The rats of the firstexperimental group were additionally supplemented daily *per os* with a complex biologically active additive in a dosage of 1.5 g per animal. The rats of the second experimental group were fed only with toxic feed. The third group of rats served as a biological control, receiving benign mixed feed. The effectiveness of the protective action of the additive was estimated by the survival rate of rats, gravimetric body weight, clinical signs, and changes in biochemical blood factors. **Results:** Application of the complex additive consisting of product processing plant raw materials - sugar beet pulp and rapeseed lecithin (in the ratio 4:1) on the background of feeding rats with the feed, contaminated with mycotoxins (association T-2 toxin, zearalenone, and aflatoxin B1), leads to a weakening of the action of xenobiotics that are manifested by improvement of biochemical homeostasis and functional state of liver of the experimental animals. **Conclusion:** The conducted studies have shown that the use of plant dietary fibers in combination with phospholipids of lecithins on the laboratory background of mycotoxicosis results in weakening of xenobiotic action, which is manifested by improvement of biochemical homeostasis and liver functional status of experimental rats, increasing their safety, and decreasing clinical manifestations of intoxication.

**Key words:** Dietary fiber, lecithin, mycotoxicoses, phospholipids, toxicology

## INTRODUCTION

Mycotoxicoses are diseases of human and animals, caused by entering the body of vital activity products of a number of microscopic fungi - mycotoxins. At present, more than 300 mycotoxins have been allocated from fodder and food products and their number is constantly increasing. Moreover, the formation of mycotoxins is a poorly predicted phenomenon, and it is impossible to prevent their accumulation in food products completely and, therefore, to avoid entering the human body. At least 25% of all food resources are susceptible to contamination with mycotoxins or damaging effects of mold fungi, and the expansion of grain exports and imports facilitates the rapid spread of phytopathogenic fungi around the world.[1,2] At the same time, in the process of technological and culinary processing of food products, there are practically no reliable methods for their removal. Mycotoxins are extremely heat-resistant compounds that can withstand temperatures of + 100°C or more. Freezing, drying, and exposure of ionizing and ultraviolet radiation are also ineffective ways to inactivate them.

Numerous studies have shown that mycotoxins are highly toxic and many of them have mutagenic, teratogenic, carcinogenic, and immunosuppressive properties. The level

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of danger increases with simultaneous intake of two or more mycotoxins in the body, as well as their combinations with toxic pollutants - pesticides, dioxins, etc. Such interaction can not only significantly increase the toxicity of mycotoxins but also have a significant pathogenic effect on human and animal health.[3]

Thereby, along with activities aimed at preventing mycotoxins from entering the body, it is important to find ways to reduce the toxicity of xenobiotics, which are already placed in the organism. Among the most promising directions in this area can be the use of dietary fibers enhanced with hepatoprotective agents as a way to regulate the processes of toxicokinetics of foreign compounds including the stages of absorption, hepatic intestinal recirculation, biotransformation, and detoxification.[4,5]

Dietary fibers from plant raw materials are products containing particles of different sizes and characterized by a developed specific surface area; they have a considerable pore radius in comparison with the initial raw material, which determines the expediency of their use as sorbents. Dietary fibers are capable of absorbing the xenobiotics that enter the body in a multifactorial way due to:

- Mechanical sorption, for which cellulose is responsible;
- Biochemical sorption, carried out by soluble in the intestine dietary fibers with a low molecular weight (alginates, natural gums, pectins, etc.), which, dissolving in the intestine to lower molecular compounds, enter into biochemical reactions with toxic substances, which leads not only to sorption but also to neutralization of the latter;
- Biological sorption carried out by fructooligosaccharides, the mediated purifying action of which is related to the normalization of the intestinal microflora (bifido- and lactobacilli), which, in turn, neutralizes the decay products accumulating in the large intestine.[6-8]

In the conducted experiment, dietary fibers of sugar beet pulp were used, which was represented by desugared beet chips, dried up to a moisture content of 10–12%. From the biological point of view, the pulp has a number of additional advantages, because in addition to dietary fiber, it contains pectin, which in its composition is identical to the pectin of apples and citrus fruits.

Considering that liver is the target organ for mycotoxicosis, it is justified to use hepatoprotective substances that increase the resistance of liver to the action of pathogens and normalize its metabolism in conditions of a detoxifying function.

Over the past few years, positive experience in the use of preparations at hepatopathies has been accumulated that include lecithin, which is the main structural component of all cell membranes, maintaining the constancy of the internal environment of cells and participating in energy and metabolic reactions of the body. One of the most important properties of lecithin is the protection of cells from toxicants, which is partially realized by inhibiting the processes of lipid peroxidation. Phospholipids, restoring the "packing" of polyunsaturated fatty acids in the membrane of hepatocytes, reduce the access of oxygen to them, thereby reducing the rate of nucleation of the free radicals. Lecithin strengthens the walls of the cell membrane of hepatocytes, promotes regeneration of liver tissue, and also helps it to cope with the detoxification of the body from poisons and xenobiotics.[9,10]

The aim of the research was to study the protective effect of a complex biologically active additive containing dietary fiber of sugar beet pulp and lecithin in the 4:1 ratio on the model of associative mycotoxicosis reproduced on laboratory animals.

## MATERIALS AND METHODS

The object of research is a biological active additive consisting of secondary resources formed during the conversion of sugar beet and rape. Components were obtained in Krasnodar Scientific Research Institute of Storage and Conversion of Agricultural Products according to the developed technology for their production. The modification occurred due to the special preparation of the feedstock by its short-term processing in an electromagnetic field of ultrahigh frequency, which allows reducing the temperature effect on the dried material, which minimizes the loss of thermolabile micronutrients.

The experiments were carried out in accordance with the "Guidelines for experimental (preclinical) study of new pharmacological substances" (2005) and according to the experimental research protocol compiled on the basis of the "Rules for conducting preclinical research of a medicinal product for veterinary use, clinical study of a medicinal preparation for veterinary use" (2018).[11]

According to the study protocol, the experimental modeling of chronic associative mycotoxicosis was performed on 30 male rats with an average body weight of 187.1 ± 1.8 g, divided into three groups of 10 individuals each. In the experiment, we used animals, which passed the quarantine regime and did not have external signs of disease. To obtain statistically reliable results, the groups were formed according to the principle of paired analogs. The studies were carried out in accordance with the principles set forth in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986).

The essence of the method for the reproduction of associative mycotoxicosis was that for 30 days, the rats of the first and second experimental groups were fed with the feed (a mixture of wheat, oats, and barley) that was naturally contaminated with mycotoxins. The laboratory studies carried out with the help of enzyme immunoassay

determined the following mycotoxin concentrations: T-2 toxin - 0.165 mg/kg (exceeding the maximum permissible level in 1.6 times), zearalenone - 0.038 mg/kg, and aflatoxin B1 - 0.001 mg/kg. The toxicity of the feed used in the experiment is confirmed by toxicological analysis on infusorians (*Paramecium caudatum*).[12]

The rats of the first experimental group were additionally supplemented daily *per os* with a complex biologically active additive in a dosage of 1.5 g per animal in a solid form (bolus), which was prepared before each feeding. The used dosage was previously determined in the experiments on the calculation of the effective dosage and on the basis of the pharmacodynamic parameters of the additive.

The rats of the second experimental group were fed only with toxic feed. The third group of rats served as a biological control, receiving benign mixed feed. In addition to the grain components of the feed (wheat, oats, and barley), the animals were fed with juicy fodders consisting of apples and pumpkin with free access to water.

The effectiveness of the protective action of the additive was estimated by the survival rate of rats, gravimetric body weights, clinical signs, and changes in biochemical blood factors characterizing the main pathological syndromes observed in liver lesions: Cytolysis was estimated by activity in serum of aminotransferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]); disturbances in the dynamics of bile were estimated in terms of alkaline phosphatase (ALP), bilirubin, and cholesterol.

Biochemical blood indices were determined on an automatic chemical analyzer Vitalab Selectra Junior with software version 1.0 (manufactured by Vital Scientific N. V, Netherlands) equipped with a thermostat, a photometer, and a processor, in which an algorithm of carrying out 40 biochemical tests was programmed. Reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany) were used for the work on the analyzer.

Clinical control was carried out daily according to the following criteria: General condition of animals, peculiarities of their behavior, intensity and nature of motor activity, condition of hair and skin, color of visible mucous membranes, and reaction to external stimuli. Weighing of rats was carried out 3 times in dynamics (background, on the 15th and 30th days of the experiment).

Blood samples for biochemical analysis were selected from five animals from each group after 15 days from the start of the experiment and 1 day after the last application of the complex biologically active additive.

At the end of the studies, five rats were withdrawn from the experiment from each group of animals (experimental and control), which were euthanized by etching with ether in accordance with the principles of bioethics. At necropsy of the animals, the macro- and micro-structure of the internal organs of the experimental rats were evaluated to study the pathogenesis of intoxication after exposure of mycotoxins on the body.

The data obtained in the experiments were biometrically processed using the Student's test.

## RESULTS AND DISCUSSION

The conducted studies showed that feeding rats of the second group with fodders, contaminated with mycotoxins, already on the 5th day of the experiment led to a decrease in motor activity, increased thirst, while reducing appetite, liquefaction of feces, ruffling and dullness of the hair, and anemia of the mucous membranes. On the 21st day, one animal died. The remaining rats of this group during clinical examination had severe oppression, lethargy and drowsiness, lack of appetite, exhaustion, and weight loss.

At autopsy of the dead rat was found a hyperemia of the kidneys and intestinal mucosa with the remnants of the contents of a dark brown color as well as the enlargement of the liver with the presence of yellow-gray areas.

Among the rats of the first experimental group, additionally receiving a complex additive, the clinical signs of intoxication were less pronounced and appeared only on the 8th day of the experiment. The safety of animals in this group during the observation period was 100%.

Gravimetric studies of body weight of experimental rats are presented in Table 1.

**Table 1:** The influence of additive on body mass dynamics of laboratory rats under experimental mycotoxicosis (M±m)

| Groups | Body weight (g) | | |
|---|---|---|---|
| | Background | After 15 days | After 30 days |
| First experimental (dietary fibers+lecithin) | 184.3±2.1 | 178.6±1.1 | 187.8±0.7* |
| Second experimental (without treatment) | 191.5±1.4 | 173.7±0.9 | 168.9±2.9 |
| Third control (healthy animals) | 185.5±1.8 | 191.8±1.2 | 200.2±2.5 |

The differences are significant (*$P \leq 0.05$) in comparison with animals receiving mycotoxins without therapy

As a result of the analysis of the data, it was determined that the use of preventive therapy of combined mycotoxicosis in the first experimental group neutralized the loss of body weight of animals. In the first phase of the experiment (after 15 days), the weight differences of rats in comparison with background indices were 3.2%, and by the end of the experiment, positive growth dynamics was recorded with an increase in initial body weight by 1.9%.

In the second group (without treatment) by the 15th day of studies, a dynamic decrease in the body weight of the experimental rats by 10.2% was registered, and by the end of the experimental period, the decrease was 11.8%.

In the biochemical study of blood, it has been found out that the intake of mycotoxins leads to significant changes in a number of biochemical blood indices [Table 2].

The toxicity of the association of T-2 toxin, zearalenone, and aflatoxin B1 on the animal's organism was manifested by pronounced changes in the activity of enzymes - markers of the functional state of liver. In the second experimental group (without treatment) in comparison with intact animals, an increase in AST activity by 46% was recorded by the middle of the experiment. At the end of the experiment, the difference between the groups was 24.8%. ALT activity increased more significantly - on the 15th day, it increased in 3.7 times and on the 30th day, it increased in 2 times compared with the data obtained in the control group of rats.

The inclusion of a biologically active additive in the diet contributed to a significant weakening of the effect of mycotoxins on liver. Among the rats of the first group, the activity of AST was lower by 18.2% ($P \leq 0.05$) by the middle of the experiment, and by the end of the studies, it was lower by 16% ($P \leq 0.01$), nevertheless, exceeding the values of the analog rats of the control group. The same dynamics was observed in the ALT: The difference on the 15th day of observations was 44.7% ($P \leq 0.001$), on the 30th day of studies, it was 3 times higher ($P \leq 0.001$) relative to the rats of the second group, while remaining higher than the corresponding index in intact animals.

Among animals receiving a complex additive, the concentration of total bilirubin exceeded the indicators of the rats of the first group by 42.2% by the end of the experiment ($P \leq 0.05$). The level of ALP in the first group was higher by 40.7% at the end of the experiment and almost 2 times higher in the second group ($P \leq 0.01$). A similar situation was with the level of cholesterol, which was confirmed by its lower concentration in rats receiving the additive, relative to the animals of the second group, with a difference of 33.3% ($P \leq 0.001$) in the last period of the study. A similar biochemical picture indicates the presence of a cholestatic syndrome caused by a violation of the bile excretory function of liver and damage of bile ducts (intrahepatic cholestasis).

The entry of mycotoxins in the animal body caused a violation of the protein-genetic function of liver, which was confirmed by a decrease in the content of total protein in the second group by 30.4% in relation to healthy rats. The use of dietary fiber and lecithin phospholipids allowed minimizing the development of protein disorders - the difference in protein in the first group was 10.2%.

The obtained results of biochemical studies of the blood serum of the rats make it possible to establish that when the mycotoxins enter the living organism, an increase in the activity of aminotransferases occurs, which proves the damage of hepatocyte membranes, as well as the death of liver cells under the action of xenobiotics, leading to the release of intracellular substances into the blood and lymph. This process is accompanied by intrahepatic cholestasis and a violation of the protein-synthesizing function of liver, while the use of plant fibers in combination with lecithin phospholipids improves the parameters of biochemical constants of homeostasis and liver functional status of experimental rats against associative mycotoxicosis.

**Table 2:** The influence of additive on biochemical blood indicators of laboratory rats under experimental mycotoxicosis (M±m; *n*=5)

| Indicators | First experimental (dietary fibers+lecithin) | | Second experimental (without treatment) | | Control (intact) | |
|---|---|---|---|---|---|---|
| | Days of experiment | | | | | |
| | 15 | 30 | 15 | 30 | 15 | 30 |
| AST, IU/L | 96.0±5.7* | 88.0±3.4** | 113.3±3.7 | 102.1±5.4 | 77.6±4.3 | 81.7±2.6 |
| ALT, IU/L | 87.3±3.4*** | 91.7±1.25*** | 126.3±7.2 | 273.5±5.5 | 61.4±5.8 | 64.5±4.1 |
| ALP, IU/L | 316.7±10.6** | 299.3±8.3** | 383.0±6.3 | 412.5±6.5 | 192.0±4.4 | 212.7±8.5 |
| Total bilirubin, µmol/L | 6.9±0.11 | 6.4±0.10* | 7.6±0.04 | 9.1±0.02 | 6.2±0.08 | 6.1±0.11 |
| Total protein, G/L | 64.9±0.47* | 69.6±0.34** | 62.9±1.06 | 58.8±0.73 | 69.9±0.51 | 76.7±0.16 |
| Glucose, mmol/L | 7.3±0.39 | 8.1±0.18* | 7.1±0.69 | 5.9±0.42 | 8.7±0.31 | 9.4±0.87 |
| Cholesterol, mmol/L | 1.5±0.05*** | 1.6±0.01*** | 1.4±0.06 | 1.2±0.04 | 1.6±0.08 | 1.7±0.06 |

The differences are significant (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) in comparison with animals receiving mycotoxins without therapy, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

At the end of the experiment, the animals of the experimental groups were euthanized by the method of euthanasia (with observance of the principles of bioethics) for revealing the pathoanatomical changes in the internal organs.

During external examination, all rats of the second experimental group were diagnosed with cyanosis of visible mucous membranes, ruffling, and dullness of the hair. Macroscopic examination revealed pathological changes, manifested to varying degrees by hemorrhagic inflammation of the gastric mucosa, thin and thick intestine, edema, and fullness of lungs. The liver was enlarged in size, flabby, dark red, or sand colored, with visible gray areas of necrosis in some places.

The inclusion of a biologically active additive in the diet made it possible to reduce significantly the effects of intoxication (pronounced macroscopic organ changes were observed in only 30% of rats).

Histological examination of the liver in rats in the group without treatment revealed massive progressive necrosis of tissues in most cases [Figure 1].

Large sections of the liver parenchyma were involved in the process, the beam structure was poorly expressed, foci of hepatocyte necrosis in the form of a nuclear-free mass were visible, their nuclei were in the state of karyorrhexis and karyolysis, the contours of the cell membrane were indistinct. Areas of hemorrhages in the form of congestive accumulations of blood in the vessels and intercellular space of the liver parenchyma were visible. Necrosis was centrolobular, with the spread of the pathological process from the center to the periphery. Lipodystrophy was noted in hepatocytes, which is characterized by cells having fatty droplets of various sizes in the cytoplasm, partially or completely displacing the nuclear remains to the cell membrane. There were small areas of inflammatory reaction, represented by the proliferation of lymphocytes.

Animals of the first experimental group receiving the additive showed submissive necrosis of liver [Figure 2]. The lobular structure was preserved, cells of the healthy tissue had clear boundaries and the prominent beam structure, nuclei were located in the center of cells, the cytoplasm was homogeneous, the cell membrane was integral. However, small areas of necrosis, nuclei of hepatocytes in the state of karyopyknosis and karyolysis were detected; the border between the cells was indistinct. Liver cells at the border of healthy and damaged tissue were in the state of lipodystrophy and had small inclusions of fat drops. The inflammatory reaction was poorly expressed.

Earlier, studies on laboratory animals found out that lecithin obtained from rapeseed oils show antioxidant properties.[13] This ability occurs due to a significant number of such phospholipids, as phosphatidylethanolamines and phosphatidylserines, as well as tocopherols, which exhibit antioxidant properties.[14] In this connection, the complex biologically active additive has a hepatoprotective effect on

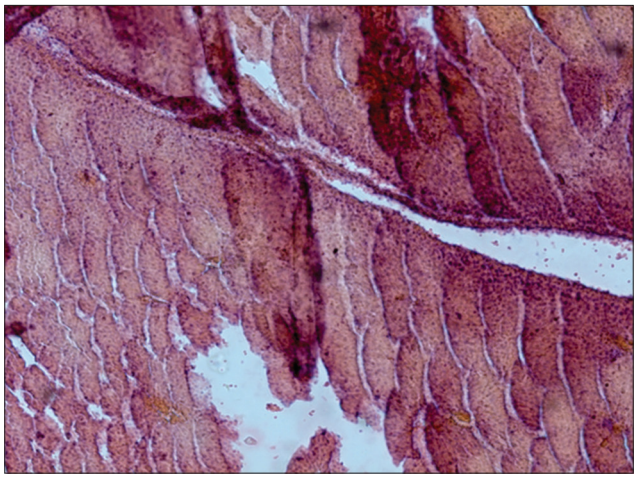

**Figure 1:** Tissue of the liver of rats in the group without treatment

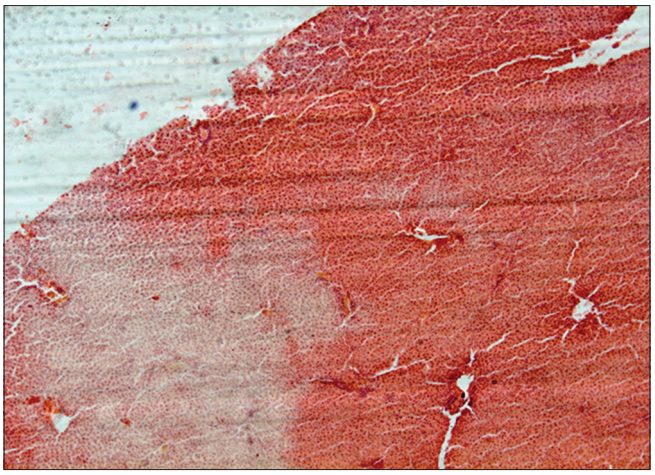

**Figure 2:** Tissue of the liver of rats in the group with the use of additives

the metabolic level with a seed of mycotoxins. This happens due to the response of the body to external negative effects by a condition called oxidative stress (an increase in oxidative reactions in organs and tissues), induced by harmful environmental factors, accompanied by an increase in the concentration of free radical forms of metabolites that disrupt the metabolic and energy processes in the body and lead to the destruction of cell membranes, and pathologically altered functions of cells including hepatocytes.[15,16] Potentiation of the hepatoprotective effect in the additive is achieved by the use of plant fibers from sugar beet, additionally including pectin and cellulose. These components have the ability to form complexes with xenobiotics and remove them from the body and also to improve the functional state of the gastrointestinal tract due to the prebiotic properties.[17]

## CONCLUSION

Thus, the conducted studies showed that entering of mycotoxins into the body of rats in the association leads to

the development of cytolytic syndrome (increase content of AST in blood), cholestatic syndrome (increase in activity of ALP and bilirubin concentration), and hepatodepressive syndrome (decrease in total protein) of liver damage. The use of the additive containing plant fiber and phospholipids on this background results in a weakening of the action of xenobiotics, which is manifested by an improvement in the biochemical homeostasis, as well as the functional and structural state of liver of the experimental rats, increase in their safety, and decrease in the clinical manifestations of intoxication.

On the basis of the obtained data it can be concluded that the complex of phospholipid and polysaccharide substances presented in the additive can be recommended for use in the treatment of toxic liver damage of animals.

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