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HISTOLOGICAL AND MORPHOLOGICAL CHANGES OF RATS' THYMUS UNDER THE INFLUENCE OF AFLATOXIN B1

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The issue of investigating dangerous influence of mycotoxins (toxic substance produced by mushrooms/ fungi) is quite timely. Food or feed polluted by them cause various infections – mycotoxicoses. Mycotoxins influence organism in many ways. Immune system suffers particularly severely. The damage of the latter leads to high danger of virus and bacterial infection risk, efficiency of precautions decreases. This study aims to find out the effects specifications of Aflatoxin B1 on the morphofunctional several criteria of rats' thymus.

Summarizing the received data we come to a conclusion that the response of rats' thymus during the first 15 days may be described as an adaptive-compensatory (migration of lymphocytes increase), but during the second month retrospective, reactive changes begin to demonstrate in the organ, witness the increasing mast cells and the reduction of volumetric ratio cortex of lobes.

Aflatoxin B1 – rats' s thymus – histological changes– mast cells

Միկոտոքսինների՝ սնկերից արտադրվող թունավոր նյութերի վնասակար ազդեցության ուսումնասիրության հիմնախնդիրն արդիական է: Դրանցով աղտոտված սննդամթերքը կամ կերը պատճառ է դառնում օրգանիզմների զանազան ախտահարումների՝ միկոտոքսիկոզների: Միկոտոքսինները բազմակողմանի ազդեցություն ունեն օրգանիզմի վրա: Առանձնապես խիստ տուժում է իմունային համակարգը: Վերջինիս վնասվելու հետևանքով մեծանում է վիրուսային և բակտերիական վարակների վտանգը, նվազում է նախազգուշական միջոցառումների արդյունավետությունը: Սույն աշխատանքում մեր առջև խնդիր էր դրված պարզել առնետների թիմուսի մորֆոֆունկցիոնալ մի քանի չափանիշների վրա աֆլատոքսին B1-ի ազդեցության առանձնահատկությունները:

Ստացված տվյալները ցույց են տալիս, որ առնետների թիմուսի պատասխանը միկոտոքսին՝ աֆլատոքսին B1-ի սննդի հետ ներմուծմանը առաջին 15 օրվա ընթացքում կարող է բնութագրվել որպես հարմարողական, փոխհատուցողական (մեծանում է լիմֆոցիտների արտագաղթը), իսկ երկրորդ ամսվա ընթացքում օրգանում սկսում են գերակշռել հետադիմական, ռեակտիվ փոփոխությունները, որոնց մասին վկայում են պարարտ բջիջների բանակության մեծացումը և բլբակների կեղևային նյութի հարաբերական ծավալի փոքրացումը:

Աֆլատոքսին B1 – առնետների թիմուս – հյուսվածաբանական փոփոխություններ – պարարտ բջիջներ

Проблема изучения патологического влияния микотоксинов – токсических веществ, продуцируемых плесневыми грибами, является весьма актуальной. Поражение пищи или корма микотоксинами является причиной развития различных патологических состояний – микотоксикозов. Микотоксины имеют многостороннее влияние на организм. Особенно поражается иммунная система, вследствие чего повышается риск вирусных и бактериальных инфекций, снижается эффективность профилактических мероприятий. В данной статье рассматриваются особенности влияния афлатоксина B1 на некоторые морфофункциональные показатели тимуса крыс.

Полученные данные показывают, что в тимусе крыс, получавших в течение 15 сут загрязненный афлатоксином В1 корм, наблюдаются адаптивно-приспособительные процессы, которые проявляются в увеличении миграции лимфоцитов из тимуса. Гистоморфологическое исследование тимуса крыс, получавших в течение двух месяцев загрязненный корм, выявило ряд деструктивных и дегенеративных изменений, о чем свидетельствуют увеличение количества тучных клеток и уменьшение относительного объема коркового вещества долек тимуса.

Афлатоксин В1 – тимус крыс – гистологические изменения – тучные клетки

Mycotoxins are low molecular weight molecules produced as secondary metabolites by filamentous fungi that can be found as natural contaminants in many foods and feeds. These toxins have been shown to have adverse effects on both human and animal health, and are cause of significant economic losses worldwide [9]. The most widespread mycotoxins, contaminated food and environmental are Aflatoxins, Ochratoxins and Fuzaritoxins. These mycotoxins are produced by mold fungi of sorts *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria*. The toxins produced by these fungi can cause considerable health risks and significant economic losses due to fungal deterioration of the agricultural commodities [9, 10]. These substances generally contain a few hundred species of toxic components with the use of contaminated foods or feeds in human and animal organisms can cause various acute and chronic disorders [4.5].

In the literature there are the data, testifying that in the conditions of mycotoxins influence chromosomal damages, inflammatory and necrotic processes, a hypertrophy of hepatic ducts, quantity of lymphocytes increase in activity of some enzymes in a liver, reduction of weight of a spleen and thymus mast cell necrosis, increase in a susceptibility of animals to virus, bacteriological and parasitic diseases, increase in relative weight and necrosis of kidneys, nephrites are observed [1, 7].

Pathologic changes in the liver, kidney and brain have been observed in case of Aflatoxins organism injection also in case of joint injection with Ochratoxins have been observed cerebral hypoplasia, structural disruptions of skull bones and eyeretina [11]. For the detection of molecular defects of immune response under the influence of Aflatoxins currently are also observed biotic and antibiotic cytokines as well as changes in the level of expression of the macrophage membrane differentiating receptors [2].

Taking into consideration the fact that cytological and histological patterns of reactive changes in immune system organs are still insufficiently explored under the influence of mycotoxins so our task was to explore histological changes of rats' thymus under the influence of Aflatoxin B1.

This study aims to find out the effects specifications of Aflatoxin B1 on the morphofunctional several criteria of rats' thymus.

Materials and methods. 32 mature rats received Aflatoxin B1 with the feed (body weight of 150-200 grams), in fact their thymus was used as material for experiments. All animals were divided into groups, which were different from one another by the duration of experiment and mycotoxins were imported with feeds (tab. 1).

Animals were getting Aflatoxin B1 everyday with their feed (0,0257MG). The animals were divided into 3 groups. Animals belonging to the first group were being fed by a contaminated feed withing 15 days, animals of second group got contaminated feed 30 days, and the third group of animals within tow months. Animals' thymus served as a controller which had been fed with non contaminated feed. Under general anesthesia the animals were weighed and were killed by beheading and then their thymus was taken and their sampls for histological processing.

Thymus samples were fixed in the solutions of Bouin and neutral buffered 10% Formalin solution (Sigma Aldrich). Fixed material was subjected to histological processing and enclosed in paraffin. 5-6 µm paraffin serial sections were staining with solutions of Hematoxylin and eosin,

Table 1. Experimental animals division of groups

Mycotoxins	Duration of the experiment	Number of animals
Controller		8
<i>Aflatoxin B1</i>	15 days	8
	1 month	8
	2 months	8

Giemza, May-Grunwald according to Papenheim and toluidine blue (Sigma Aldrich). Preparats, staining by Hematoxylin and eosin, was decided dimensional correlation between cortex and medulla materials in the thymus lobes by stereological method, and then the number of mast cells was calculated on a certain surface. The received digital data were subjected to statistical analysis. The reliability of the differences between the values obtained in the experimental and control materials were determined using the computer program "Statistica 8".

Results and Discussion. After import of Aflatoxin B1 by feed, on the 16th day experiment microscopic study of thymus showed a decrease in the number of lymphocyte in the thymus. This is known in the literature under the name of aplasia. In the lobes of thymus especially in the cortex the lymphocytes are noticeable more split presented. As a result epithelioreticular cells which form organ lacy basis become very visible on cuts. As a result of the decrease in the number of lymphocytes in some parts of the lobes the difference between the cortex and medulla almost became unobserved, which is always well expressed in the control animals thymus (fig.1).

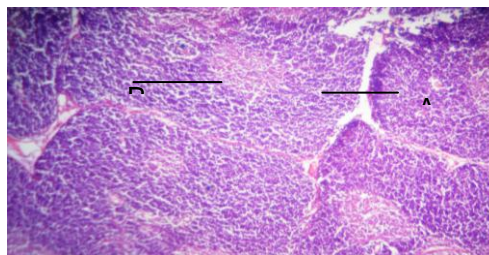


Fig. 1. The cut of thymus of rat.
A-Cortex, B- Medulla: Staining with hematoxylin and eosin. Zoom-200x

The loss of lymphocytes in the thymus is probably due to high activity coming out of lymphocytes from thymus. We can say, under the influence of toxins, the thymus provides more lymphocytes to the peripheral organs of the immune system in order to provide immunogenesis. Noteworthy is the fact that on the sections of the thymus were often encountered postcapillary venules which walls were composed of cuboidal cells instead of flat plates during that time of the experiment. It is known that the endothelial wall of postcapillary venules of blood-forming organs is undergoing similar changes during the active transport of leukocytes.

Histological condition of thymus of the rats, fed by contaminated feed by Aflatoxin B1 within 30 days, can be described in higher expression of aplasia. The number of lymphocytes more decreased in the organ. In addition, reactive condition of body is evident. Firstly, we judge about it based on a significant increase in the number of mast cells. In the last period of our observations, after animals had been fed by Aflatoxin B1 contaminated feed within 60 days aplasia and a large number of mast cells were retain in the thymus compared with the controller.

As it is known the mast cells are composed of one of the specialized cell population of internal environment tissue. They actively participate in inflammation, immunogenesis process, blood clotting, blood circulation process- contributing to the

maintenance of local homeostasis. These cells perform their protective and regulating function through special mediators. They play an important role in the regulation of the migration of the cells from the blood vessels to the tissues of the affector cells and also contribute to selective communication between them and endothelial cells-adhesion. Mast cells are placed in the thymus mainly under the capsule and in the interstitial connective tissue. They are remarkable with considerable polymorphism which is expressed in the diversity of their size and shape, also in different densities of granula in cytoplasm. The mast cells in the thymus of rats are quite large cells, the large granuls which are in cytoplasm are gifted by metachromasy feature. On the tollodin blue preparations are colored by dark purple. Due to the thick layers of the granuls, cell nucleus is usually concealed.

As you can see from the table below (tab.2) after rats were fed by contaminated feed by Aflatoxin B1 within 30 days the number of mast cells do not undergo significant changes in the thymus.

Table 2. Changes in the number of mast cells in the rats' thymus which were fed by contaminated feed with Aflatoxin B1

Duration of the experiment	The number of mast cells (M±m)	P
Controller	13.33 ± 4.7	
15 days	14.80 ± 3.61	p>0.5
30 days	10.48 ± 4.91	p>0.5
60 days	21.55± 2.36	p<0.5

In the next period of experiment, after rats were fed by contiminated feed by Aflatoxin B₁ within 60 days the number of mast cells increases significantly in the thymus. They appear in large groups in the tunica adventitia, in the interstitial connective tissue and in the cortex of lobe. This is evident by increasing the activity of mast cells in the organ within the specified period of experiment and also the presence of vascular reactions under the influence of mediators produced from mast cells (fig. 2).

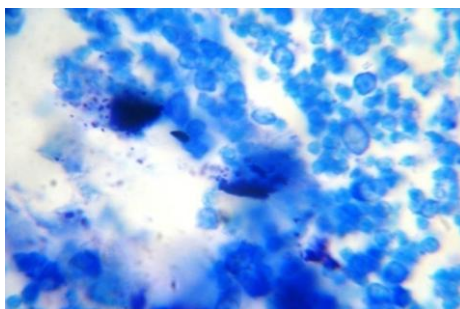


Fig. 2. Mast cells in the cortex of the lobes of thymus. Staining according to Papenheim. Zoom-1000x.

After importing Aflatoxin B1 with feed in order to judge about morphofunctional changes of thymus during different time of experiment the second criterion we observed was the volumetric ratio between medulla and cortex in the lobes.

During the first month of experments the volumentric ratio between medulla and cortex ins't changed, despite the presence of aplasia, decrease in the number of lymphocytes.

But in the last period of our observation, the animals, after being fed by mycotoxins contaminated feed within two months, the volume of cortex of lobes has decreased, moreover, that decreaseion is statistically reliable (fig.3).

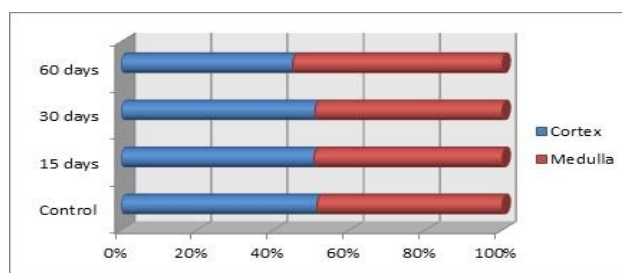


Fig. 3. Changes in volumetric ratio between medulla and cortex of lobes in the thymus of rats which were fed by contaminated feed with Aflatoxin B1 during the different time of experiment

Thus, summarizing the received data we come to a conclusion that the response of rats' thymus during the first 15 days may be described as an adaptive-compensatory (migration of lymphocytes increase), but during the second month retrospective, reactive changes begin to demonstrate in the organ, witness the increasing mast cells and the reduction of volumetric ratio cortex of lobes.

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