MEDICAL SCIENCES

EFFECT OF ACUTE INTOXICATION WITH AFLATOXIN B1 ON THE ANTIOXIDANT SYSTEM IN RED BLOOD CELLS AND LUNG CELLS OF WHITE RATS

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Abstract. Contamination of agricultural products with mycotoxins is a widespread phenomenon that is manifested in many countries. Among mycotoxins, which significantly affect human and animal health, aflatoxins produced by *Aspergillus* micromycetic fungi (mainly *A. flavus*) are of serious concern because of their high toxicity and carcinogenicity. The aim of our study was to investigate the effect of aflatoxin B1 (AFB1) on intracellular metabolism in laboratory animals. The dynamics of the lipid peroxidation (LPO) and activities of antioxidant system enzymes (superoxide dismutase, glutathione peroxidase, and glutathione reductase) in lung cells and erythrocytes of white rats were analyzed within 14 days after administration of AFB1 (dose 1/10 LD50). The results of the study have shown the activation of the LPO processes and a decrease in the activity of enzymes of the antioxidant system in the cells of rats of the experimental groups. In general, the obtained results suggest the development of oxidative stress in the lungs cells and erythrocytes of animals under the influence of aflatoxin B1.

Keywords: aflatoxin B1, oxidative stress, lipid peroxidation, antioxidant system

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Introduction

Mycotoxins are a group of natural toxins produced by mould micromycetic fungi, which cause toxic reactions in humans and animals if they are ingested with contaminated food, inhaled, or absorbed through the skin. The ingestion of food and feed contaminated with toxigenic moulds is the main pathway of mycotoxin entry into human and animal body. The most important groups of mycotoxins are produced by moulds of the *Aspergillus*, *Penicillium* and *Fusarium* genera and include compounds such as aflatoxins, ochratoxins, fumonisins, trichothecenes, etc. (*Bennett and Klich, 2003; Antonyak, et al., 2013; Sobral et al., 2018*). Intoxication caused by mycotoxins is accompanied by negative changes in metabolic processes that contribute to the development of the pathological state of the body. Mycotoxins can cause economic losses in animal husbandry and significantly reduce the quality of animal products (*Dagnas and Membre, 2013; Skládanka et al., 2011*). According to the FAO (Food

and Agriculture Organization), almost 25% of the world's crops yields each year are contaminated with mycotoxins, whose concentrations in agricultural products largely depend on annual changes in weather and other environmental factors.

Among mycotoxins that significantly affect the functions of organs and systems of humans and animals, aflatoxins produced by micromycetes of the genus *Aspergillus* (*A. flavus*, *A. parasiticus* and, in rare cases, *A. nomius*), are of serious concern due to their high toxicity, carcinogenicity, mutagenicity and teratogenicity (*Jeffrey et al., 1996; Du et al., 2019*). The most distributed and hazardous of these substances is aflatoxin B1 (AFB1), which is often referred to as the most potent natural carcinogen (*Antonyak, et al., 2009, 2015; Fang et al., 2018*).

Aflatoxin B1 is capable of causing strong damage to the animal and human body over a short period of time. This toxin can contaminate animal feed and human food because of the proliferation of micromycetic fungi on natural substrates (seeds of cereals, legumes, cotton and peanuts, nuts, dried fruits, spices, etc.). Aflatoxin B1 is characterized by hepatotoxic, nephrotoxic, carcinogenic, mutagenic, teratogenic, immunosuppressive effects. Even at low concentrations, AFB1 reduces productivity, growth intensity and animal health (*Eraslan et al., 2017*). Depending on the level of AFB1 entry into the body, this toxin causes poisoning known as acute and chronic aflatoxicosis (*Bennett and Klich, 2003; D'Angelo et al., 2007; Wang et al., 2016*). In this regard, the biological effects and mechanisms of action of AFB1 in human and animal body are the subject of detailed research.

Among the intracellular effects of AFB1, an important role is played by its ability to stimulate the formation of reactive oxygen species (ROS) and lipid peroxidation (LPO) processes, causing the development of oxidative stress in various types of cells (*Thomas et al., 1996; Eraslan et al., 2017; Rotimi et al., 2018*). In a number of experimental studies, the effects of AFB1 on the activities of enzymes of the antioxidants system in liver, kidney and other animal organs have been shown (*Antonyak et al., 2015; Souto et al., 2018*). However, the effects of AFB1 on the respiratory system and the gas transport function of blood in humans and animals have not been completely studied. Therefore, changes in prooxidant-antioxidant balance in lung cells and in erythrocytes in conditions of intoxication of the body with aflatoxin B1 are of great interest.

The purpose of the work was to investigate the effect of aflatoxin B1 on the process of lipid peroxidation and the activities of the antioxidant system enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase) in lung cells and red blood cells of white rats in conditions of a single AFB1 injection in a dose of 1/10 LD_{50}.

Materials and methods

Experiments were carried out on adult white male rats weighing 180-200 g. During the experiment, the animals were kept under standard vivarium conditions with maintenance of food and drinking regimens at the level recommended by the standards for keeping laboratory animals. The rats were divided into three groups: control (K, n = 14) and two experimental groups (E1, E2) with 7 animals in each group. Animals of the experimental groups received a single injection of AFB1 solution in olive oil at a dose of 0.5 mg/kg of weight (which is 1/10 of LD50), and the animals in control group received olive oil injection in an appropriate volume. The studies were conducted at the 7th and 14th day after administration of AFB1. Animal euthanasia was conducted in accordance with the biotic requirements for animals that comply with the Law of Ukraine "On the Protection of Animals from Cruel Treatment"

(February 21, 2006) in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (*Strasbourg, 1986*) and the general ethical principles of experiments on animals approved by the National Congress on Bioethics, held in Kyiv, 2001.

The lung samples were taken immediately after euthanasia, cooled to a temperature of 1-3°C in a physiological solution, dried with filter paper, and then shredded with scissors and homogenized in 0.05 M tris-HCl buffer (pH 7.5) with addition of 0.25 M sucrose using the homogenizer MPW-324 (Poland). The weight ratio of the tissue to the buffer volume was 1:9. The resulting homogenates were centrifuged at 10,000 g for 30 minutes at a refrigeration centrifuge MLW-T23D (Germany) using supernatant fluid for studies.

Peripheral blood was collected in heparin test tubes, the plasma was separated by centrifugation, and the erythrocytes were washed three times with 0.9% NaCl solution, each time centrifuging a cell suspension at 3000 g for 5 minutes. Hemolysates were prepared by freezing and thawing of red blood cells, followed by centrifugation at 10,000 g for 15 min.

In homogenates of lung cells and hemolysates, the concentration of products that interact with thiobarbituric acid (TBA-active products) and the activities of the antioxidant system enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase) were analysed.

Superoxide dismutase activity in lung cell homogenates and red blood cell hemolysates was analyzed, taking into account the level of inhibition by the enzyme of the reduction of nitrosine tetrazolium in the presence of NADH and phenazine methsulphate (*Prokhorov, 1982*). Glutathione peroxidase activity was determined by the level of oxidized glutathione accumulation (GSSG), glutathione reductase – by the oxidation rate of NADPH molecules. The obtained results were processed statistically using the variation statistics methods (*Lapach et al., 2001*).

Results and discussion

The results of study show that after administration of AFB1 to animals, the level of TBA-active products in pulmonary cell homogenates increases at the 7th and 14th days of the experiment by 83.7% and 97.1%, respectively, and by 55.8% and 89%, respectively, in red blood cell hemolysates ($P < 0,05$-$0,01$) (Fig. 1). The obtained results indicate an increase in the intensity of lipid peroxidation in studied cells under the influence of aflatoxin B1. As it is known, intensification of the LPO process can lead to harmful effects, such as oxidative damage to the membranes, inhibition of the catalytic activity of enzymes, etc. (*El-Beltagi and Mohamed, 2013*).

Under conditions of enhanced LPO processes, the functional activity of intracellular protective systems plays an important role. One of the most important protective systems is an antioxidant system, which is represented by a complex of non-enzyme antioxidants and specialized enzymes catalyzing the processes of ROS detoxification. The enzymes of antioxidant system such as superoxide dismutase (SOD) and glutathione peroxidase are known to scavenge ROS and protect the cells against deleterious consequences of lipid peroxidation. At the same time, glutathione peroxidase needs the molecules of the reduced glutathione (GSH) for its catalytic activity. The process of glutathione reduction is catalyzed by the enzyme glutathione reductase, which is an important component of the antioxidant system. Thus, components of the antioxidant system are involved in the regulation of the free radicals formation and the elimination of products of lipid peroxidation *(Antonyak et al.*

2015). In order to evaluate the dynamics of antioxidant enzyme activities in the conditions of acute intoxication by AFB1, the following stage of our experiment was aimed to evaluate the activities of SOD, glutathione peroxidase and glutathione reductase in the lung cells and erythrocytes of rats.

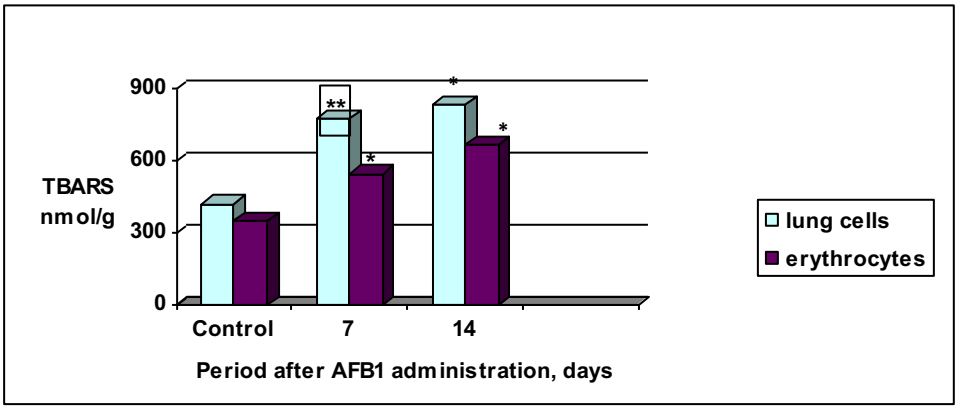

Fig. 1. Effect of AFB1 on the concentration of TBC-active products in lung and red blood cells of rats

Note: in this and following figures *, **, *** – statistically significant differences between control and experimental groups of animals: *–P<0.05; ** – P<0.01, *** – P<0.001.

The results of the study show that the activity of all three analyzed enzymes of the antioxidant system in lung and red blood cells of rats is suppressed under the influence of aflatoxin B1. In particular, during the 7th and 14th days of the experiment, superoxide dismutase activity in lung cells decreased by 26.2% and 42.5%, respectively (P<0.05-0.01), and in erythrocytes by 44, 1% and 55.9%, respectively (Fig. 2). The characteristic dynamics of superoxide dismutase activity can be explained by the intensive accumulation LPO products, which inhibit the enzyme activity in the cells (*El-Beltagi and Mohamed, 2013; Khlangwiset et al., 2011*).

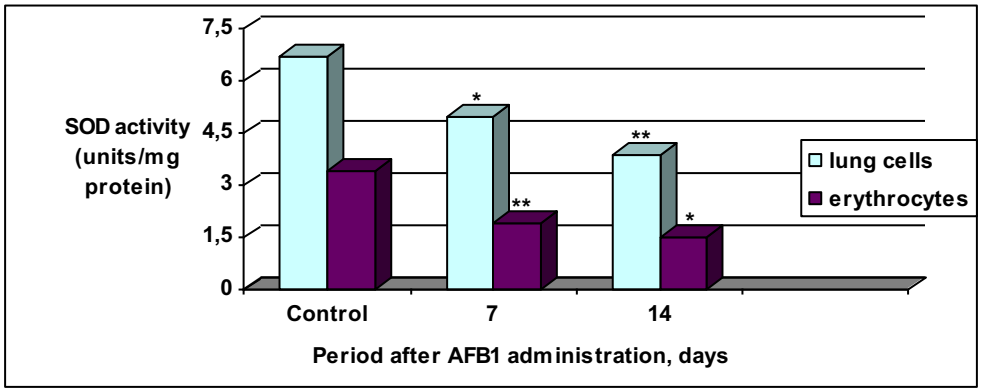

Fig. 2. Effects of AFB1 on superoxide dismutase activity in lung and red blood cells of rats

The important enzyme of the cellular antioxidant system is glutathione peroxidase, which catalyzes the process of detoxification of hydrogen peroxide (H_2O_2) formed in the superoxide dismutase reaction. Consequently, the catalytic activity of this enzyme prevents the progression of lipid peroxidation processes *(Arthur, 2000; Li et al 2014)*. The obtained results indicate that glutathione peroxidase activity in the investigated rat cells was suppressed during the experimental period by 30.1% and 23.5%, and by 46.9% and 65.2% in erythrocytes (P <0.05-0.001) (Fig. 3).

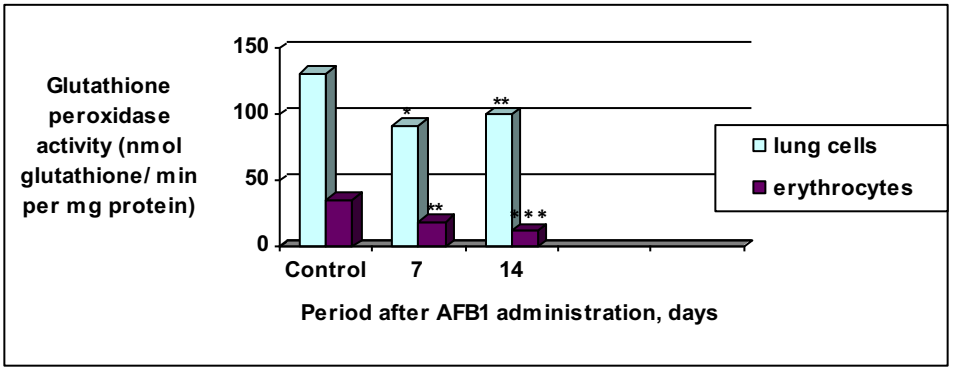

Fig. 3. Influence of AFB1 on glutathione peroxidase activity in lung and red blood cells of rats

Glutathione reductase activity in lung cells of the rats, which were injected with AFB1 was suppressed by 49.2% and 60.5%, respectively, at the 7th and 14th days of the experiment (P <0.01). In erythrocytes, the enzyme activity was reduced by 32.4% at the 7th day, and by 27.9% at the 14th day after AFB1 administration (Fig. 4).

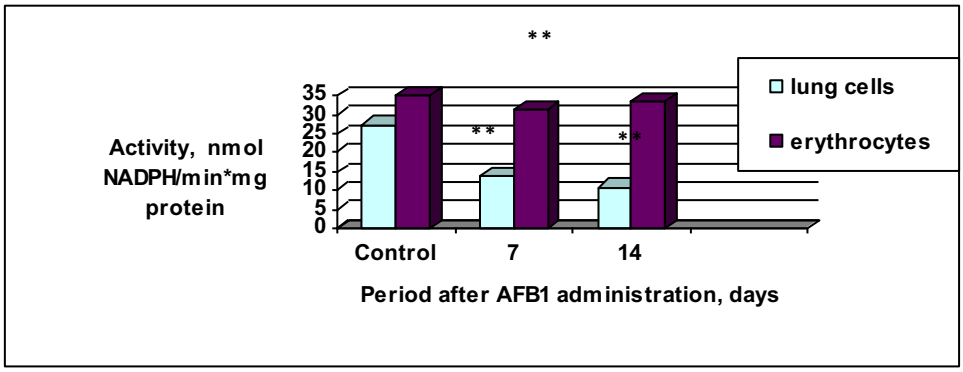

Fig. 4. Influence of AFB1 on glutathione reductase activity in lung and red blood cells of rats

The dynamics of the investigated enzymes in the cells of the lungs and erythrocytes of rats indicates the suppression of the functional state of the antioxidant system under the influence of aflatoxin B1. Under the conditions of activation of the LPO processes, inhibition

of antioxidant processes results in the development of oxidative stress and shift of the balance between the antioxidants and prooxidants in the cells. This effect leads to the accumulation of products of lipid peroxidation, which adversely affects the stability of plasma membranes, cellular metabolism and vital functions of cells (*Knipstein et al., 2015*). The adverse consequences of oxidative stress development in erythrocytes and lung cells may cause disturbances in the oxygen-transport function of the blood and pathological changes in the lung in conditions of aflatoxin B1 intoxication.

Conclusion

Under the conditions of acute intoxication with single administration of aflatoxin B1 at a dose of 0.5 mg/kg of mass (1/10 LD_{50}), the activation of lipid peroxidation processes and inhibition of the antioxidant system enzymes is observed in the lung cells and red blood cells of white rats. These effects indicate the development of oxidative stress in the lungs cells and erythrocytes after the influence of aflatoxin B1.

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