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INFLUENCE OF AFLATOXIN B_1 ON OXIDOREDUCTIVE BALANCE IN RENAL TISSUE OF RATS

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Abstract

The toxic effect of various doses of aflatoxin B_1 on renal function was studied. Experiments were conducted on randomly chosen Wistar rats. The animals were divided into four groups. Group I received 8% alcohol intragastrically. The other groups received aflatoxin B_1 in various doses. The effect of the aflatoxin on renal cells was analysed by means of determination of oxidoreductive balance and development of free radicals. The activity of antioxidative enzymes in renal tissue has decreased with an increase in the dose of aflatoxin B_1. Disturbance of oxidation balance in the kidneys confirm a toxic effect of aflatoxin B_1 on these organs.

Key words: rats, kidneys, aflatoxin B_1, antioxidative system.

The ability to produce toxic compounds is observed in numerous fungal species occurring in the natural environment. Mycotoxins are natural products of filamentous fungi and they can be toxic when introduced even in a low concentration to animals and humans. There are many reviews of the toxicity and/or genotoxicity exhibited by several mycotoxins (1, 3, 6, 10). The following mycotoxins are produced by *Aspergillus*, *Penicillium*, and *Fusarium:* aflatoxin B_1, ochratoxin, patulin, zearalenone, fumonisin and other. These pathogens cause mycotoxin accumulation in crop grain and their presence in food and fodder carries a potential risk to animal and human health. Mycotoxins may cause contamination of agricultural products during different stages of their production, storing, and processing. Mycotoxins may develop only in food, which has been attacked by mould, although the presence of mould in food does not presuppose the presence of mycotoxins. On the other hand, if agricultural products have no, or little mould, it does not mean that the products are free of mycotoxins. Some of the toxins have already been identified to be causal agents in the development of renal diseases and affect lymphocyte proliferation in pigs and cattle (14). Mycotoxins are fungal metabolites widely present in feed and food crops all over the world. Since a long-term exposure to low-levels of mycotoxins cannot be completely avoided, they are likely to be a potential concern for animal and public health. The *Aspergillus* and *Penicillium* species constitute two of the most abundant fungi found in feed and food. From the discovery of aflatoxins, mainly aflatoxin B_1 (AFB_1), their potential carcinogenic effect on number of animal species have been shown and connected with mycotoxicoses in poultry and other domestic animals. Mycotoxins are low molecular weight and heat-stable substances, which allows them to survive boiling and food processing (4, 17). Despite proper crop management, suitable storing conditions, as well as genetic engineering that aims at development of plants more resistant to diseases, mycotoxins are present in foods as natural pollutants (3). Depending on dosage and exposition time to mycotoxins, intoxication of animals may be acute, caused by high doses of mycotoxins intake, or chronic resulting from long term low dose of mycotoxin intake (10). Even small amounts of mycotoxin may cause dangerous changes in the metabolism of proteins, lipids, and carbohydrates. The most dangerous changes include disorders in nucleic acid synthesis, which is a direct cause of mutagenic and carcinogenic changes in tissues and organs. Aflatoxin contamination of food products, crop grains, and their derivatives is one of the major problems of agriculture. The significance of the problem is reflected in the number of legal regulations concerning maximum levels of aflatoxin contamination in various groups of food products. Aflatoxins are secondary metabolites, mainly produced by species of *Aspergillus flavus, Aspergillus paracisticus*, *Aspergillus nomius.* Four toxins that occur under natural conditions are divided into two groups

denoted by letters B and G. The names come from the fluorescence colour: B - blue, G - green. *A. flavus* synthesises mainly AFB_1 and AFB_2, whereas *A. paracisticus* and *A. nomius* synthesise mainly AFG_1 and AFG_2. Aflatoxin B_1 requires biotransformation to the AFB_1-8,9-epoxide by the bioactivation system and subsequent covalent binding to DNA or proteins, to exert its carcinogenic potential (12).

The aim of the study was to define the toxic effect of various doses of AFB_1 on renal function. The effect the aflatoxin on renal cells was analysed by means of determination of oxidoreductive balance and development of free radicals in renal tissues.

Material and Methods

Experiments were conducted on randomly chosen Wistar rats with the weight of 190–200 g. The animals were kept under standard laboratory conditions with permanent access to water and fodder. The room containing cages had a constant temperature of 21 ±2°C and a constant level of humidity and noise. The natural day/night rhythm of lighting was maintained. The studies began after a one-week acclimatisation period. Consent to conduct experiments on animals for scientific purposes was obtained from the Local Ethic Board prior to the initiation of the experiments. The rats were divided into five groups (eight rats in each group). Group I received intragastrically 2 ml of 8% alcoholic solution for 7 d. Group II received intragastrically 0.5 mg/kg b.w. of AFB_1 in 2 ml of 8% alcoholic solution for 7 d. Groups III and IV received in the same manner 1 mg/kg b.w. and 2 mg/kg b.w of AFB_1, respectively. Group V (control group) received intragastrically 2 ml of redistilled water. After 7 d the animals were decapitated, following the intraperitoneal administration of a sopoforic agent Vetbutal (Biowet, Poland) in the dose of 25-30 mg/kg b.w. of the active substance, and both kidneys were taken for examination. The organs were homogenised in 0.1 M Tris-HCl buffer at pH 7.4, and then centrifuged for 30 min at 5,000 rpm. In the obtained homogenates, the superoxide dismutase activity (1), glutathione peroxidase activity (12), and L-ascorbic acid concentration were measured spectrophotometrically. The obtained results were analysed statistically with the use of the Statistica 8.0. programme and tests, such as: W Shapiro-Wilka test, Kruskal-Wallis test, and Spearman's rank correlation coefficient R.

Results

The highest superoxide dismutase (SOD) activity was observed in the control group and it was on average 15.90 ±0.29 U/mg of protein. In group I, SOD activity was on average 9.12 ±0.02 U/mg of protein, in group II - 8.36 ±0.02 U/mg of protein, in group III - 6.90 ±0.02 U/mg of protein, and in group IV - 6.31 ±0.03 U/mg of protein. Statistical analysis revealed a statistically significant decrease ($P<0.05$) in SOD activity in groups II, III, and IV receiving AFB_1 in the doses of 0.5 mg/kg b.w., 1 mg/kg b.w., and 2 mg/kg b.w., respectively, in comparison with the control group V. The analysis showed no statistically significant differences in SOD activity between the control group V and group I receiving only alcohol. Fig. 1 presents a graphic interpretation of the results.

Similar results were obtained with regard to glutathione peroxidase (GPx) activity. The highest value was observed in the control group and it was on average 272.90 ±285.50 U/mg of protein. In group I, GPx activity was on average 228.30 ±2.12 U/g of protein, and in group II it was 192.50 ±1.67 U/g of protein. In group III, GPx activity was on average 130.80 ±2.21 U/g of protein and in group IV it was 123.60 ±1.95 U/g of protein. Statistical analysis showed a statistically significant ($P<0.05$) decrease in GPx activity in groups II, III, and IV, in comparison with the control group V. The analysis showed no statistically significant differences in GPx activity between the control group V and group I receiving only alcohol. Fig. 2 presents a graphic interpretation of the results.

The highest value of L-ascorbic acid was observed in the control group and it was on average 5.96 ±0.16 U/mg of protein. In groups I, II, III, and IV, the L-ascorbic acid concentration was on average 2.66 ±0.05 U/g, 3.37 ±0.08 U/g, 2.45 ±0.15 U/g, and 3.01 ±0.08 U/g of protein, respectively. Statistical analysis showed a statistically significant ($P<0.05$) decrease in the L-ascorbic acid concentration in group I receiving only alcohol, in comparison with the control group V. The analysis also showed a statistically significant decrease in the L-ascorbic acid concentration in groups III and IV receiving AFB_1 in the doses of 1 mg/kg b.w. and 2 mg/kg b.w., respectively, in comparison with the control group V. Fig. 3 presents a graphic interpretation of the results.

Discussion

Aflatoxins are a family of 20 compounds. They are mainly produced by three *Aspergillus* species: *A. flavus, A. paracisticus*, and *A. nominus.* There are four major aflatoxins: B_1, B_2, G_1, G_2, and two additional products of metabolism: M_1 and M_2. The most common food aflatoxins are B_1 and B_2, as well as G_1 and G_2. With regard to the LD_{50} index, aflatoxin toxicity is represented as follows :$AFB_1 > AFM_1 > AFG_1 > AFB_2 > AFG_2$ (7).

The degree of toxicity depends on the intensity and duration of aflatoxin exposure, age, sex, weight, and the presence of other mycotoxins (16). Bennett and Vrabcheva (3, 16) have found aflatoxins in such products as cottonseed oil, soya, peanuts, walnuts, almonds, spices, cornflakes, rice flakes, and dry fruits.

The occurrence of aflatoxins in food appears to be very important for health and economic reasons. Therefore, numerous studies have been carried out in order to determine their content in various materials and food products. A growing interest in free radicals has been observed in recent years. Free radicals are molecules of high chemical activity that oxidase each compound they encounter.

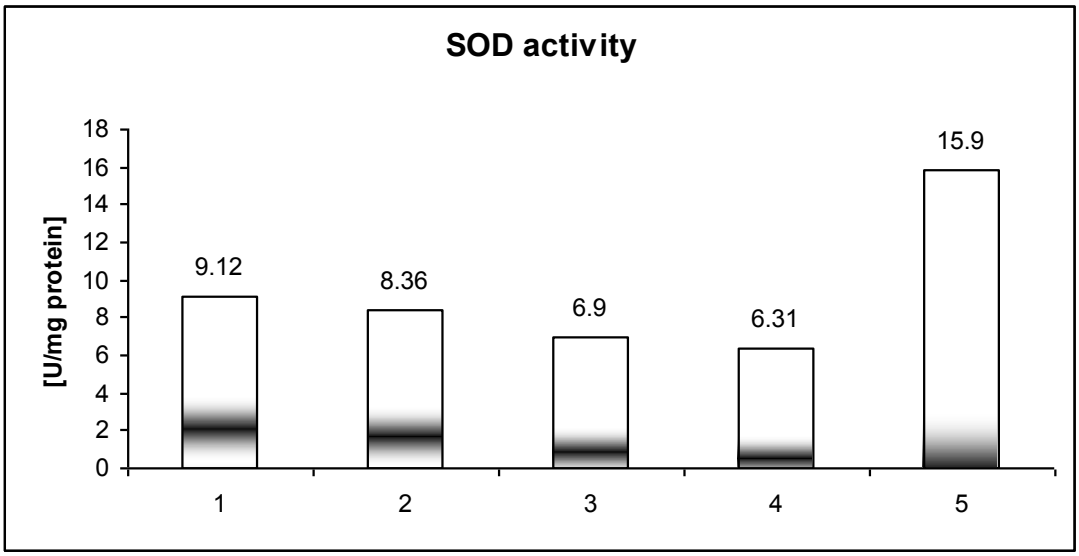


Fig. 1. SOD activity

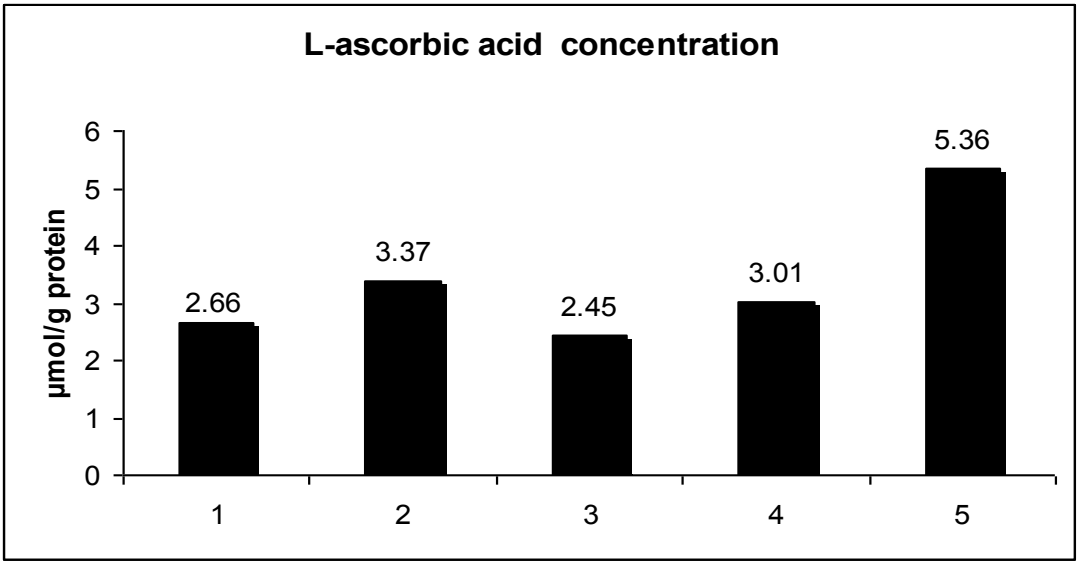


Fig. 2. GPx activity

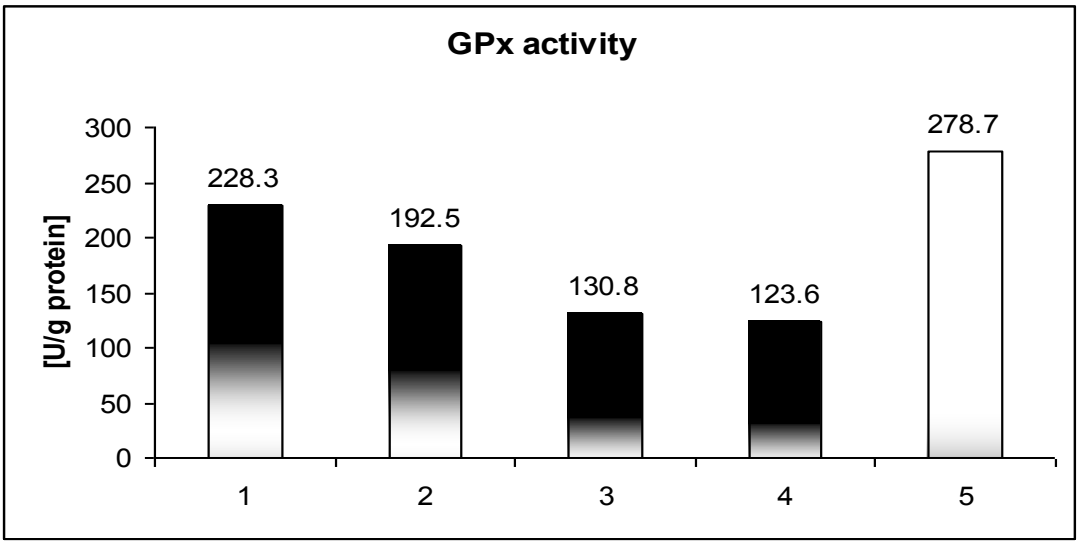


Fig. 3. L-ascorbic acid concentration

The influence of free radicals on cells depends to a great extent on their concentration and time of action. Low ROS concentration is important for physiological functions, whereas high concentration causes a toxic damage to cells resulting in their destruction (15). Free radical reactions are controlled *in*

vivo by a wide range of antioxidative mechanisms, *e.g.* enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Non-enzymatic antioxidant system is represented by such compounds as vitamins A, C, and E, as well as trace elements like selenium or zinc (5). Disturbance of the oxidation-reduction balance (ROS overproduction and/or decreased production/usage of antioxidants) leads to oxidative stress and in consequence to a destructive effect of ROS on biological structures of the body. Determination of the activity of such enzymes as SOD and GPx is used as a marker of oxidative stress. Activity of these enzymes is reduced by factors inducing oxidative stress. These enzymes constitute the first line of defence against antioxidants (2). The observed statistically significant decrease in their renal activity may indicate the AFB_1 initiation of free radical reactions in renal tissue. The role of AFB_1 in inducing oxidative stress was confirmed in studies of other authors, who also defined other markers of this process in various tissues.

Anamika Choudhary *et al.* (6) administered various AFB_1 doses to rats. The animals received intragastrically 25 and 50 mg/kg b.w of AFB_1. The aflatoxin administered for 30 d caused a remarkable increase in lipid peroxidation in rats' kidneys due to a decrease in the concentration of enzymatic antioxidants (CAT, superoxide dismutase of glutathione peroxidase) and non-enzymatic antioxidants (glutathione) compared to the control group. Other study also revealed similar changes (13).

Mathuria *et al*. (8) studied the effect of curcumin on AFB_1-induced lipid peroxidation. The level of lipid peroxidation was determined *in vitro* in liver, kidney, and testis homogenates. The results revealed that lipid peroxidation increased as the concentration of aflatoxin was elevated (2-10 mg/mL) in all homogenates.

Another experiment aiming at evaluation of the AFB_1-induced oxidative stress was carried out by Abel-Wahhab *et al.* (9). Female rats received 2 mg of AFB_1/kg b.w. Additionally, the animals received patuletin in the dose of 7.5 mg/kg b.w. or 10 mg/kg b.w. Changes typical for aflatoxicosis were observed in rats receiving only AFB_1. The activity of GPx and SOD was determined in rat renal and hepatic tissue. A remarkable increase in the activity of both enzymes was observed only in groups receiving AFB_1. Concomitant administration of AFB_1 and patuletin resulted in a remarkable improvement in all examined parameters.

All aflatoxin doses used in the study led to disturbance of oxidation balance in rats' kidneys due to development of free radicals. The studies have shown that a decrease in the activity of antioxidative enzymes in renal tissue results from oxidative stress and an uncontrolled chain reaction. ROS overproduction and the use of antioxidant reserves have led to protein oxidation, which in turn causes the modification of protein structure and function disorders. The consequences also include lipid oxidation, damage to nucleic acids, DNA chain breakage, chromosome damage, and the development of mutations leading to neoplastic changes. Oxidation affects polyunsaturated fatty acids being part of phospholipids, which constitute the main building blocks of cell membranes. Products of lipid peroxidation cause changes in physical properties of cell membrane, which may result in cell membrane damage. Thus, oxidative stress resulting from the presence of aflatoxins may contribute to, or at least be partially responsible for a renal tissue damage.

In conclusion, the activity of antioxidative enzymes in renal tissue decreases with an increase in the dose of aflatoxin B_1. Disturbance of oxidation balance in the kidneys may confirm a toxic effect of aflatoxin B_1 on these organs.

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