

THE EFFECT OF AFLATOXIN B₁ ON MEMORY PROCESSES, MORPHOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF MICE

Agnieszka Radzka-Pogoda¹⁾, Barbara Nieradko-Iwanicka²⁾

¹⁾ Doctoral School, Medical University of Lublin, Poland

²⁾ Hygiene and Epidemiology Department, Medical University of Lublin, Poland

ABSTRACT

Mycotoxins are toxic products of the metabolism of mold fungi common in the environment. Mycotoxins include aflatoxins produced by fungi of the *Aspergillus* genus. Cereal grains are most often contaminated with aflatoxins, which results from improper storage. Aflatoxins are divided into three groups: B, G and M. Toxins from the first two groups are produced by fungi and occur in the environment, the next group are metabolites of aflatoxins of group B (AFB), present in cow's milk and dairy products. The strongest effects are demonstrated by: AFB₁, AFB₂ and AFG₁, AFG₂. AFB₁ metabolism takes place in the liver with the participation of cytochrome P450. Substances are formed that are excreted in urine and bile from the body, as well as adducts that accumulate in the microsomal fraction, combining with nucleic acids and liver proteins. They have mutagenic and carcinogenic effects. The International Agency for Research on Cancer has classified AFB₁ as carcinogenic to humans. Aflatoxins have immunotoxic effects. A safe dose of mycotoxins has not been determined. Aflatoxin poisoning can be acute or chronic.

The aim of the study was to collect data on the mechanisms of the toxic effects of aflatoxin B₁ on mammals and to check whether the intraperitoneal route of AFB₁ administration can be used to model poisoning with this mycotoxin in laboratory animals.

The experiment was conducted on female Albino Swiss mice. The animals were randomly divided into 4 groups of 8. The animals were administered AFB₁ intraperitoneally for 28 consecutive days once a day.

Group I received AFB₁ at a dose of 0.1 LD₅₀ (1 mg/ kg b.w.).

Group II received AFB₁ at a dose of 0.2 LD₅₀ (2 mg/ kg b.w.).

Group III received AFB₁ at a dose of 0.5 LD₅₀ (5 mg/ kg b.w.).

Group IV received 0.9% NaCl.

After the first and last dose, a fresh spatial memory test was performed in the Y-maze. After the experiment was completed, the animals were decapitated. Blood was collected for laboratory tests: morphology and biochemistry.

No statistically significant differences were found between groups in memory, peripheral blood count, transaminases, bilirubin, cholesterol, triglycerides, gamma-glutamyl transpeptidase, creatinine. A statistically significantly lower total protein concentration was demonstrated after the experiments in the AFB₁ 1 mg/kg group (mean $\pm$ SD= 4.9g/dL $\pm$ 0.243) compared to the control group (5.428g/dL $\pm$ 0.278) ($p < 0.05$) and a statistically significant lower urea concentration in AFB₁ 1 mg/kg group (39.05mg/dL $\pm$ 0.844) compared to AFB₁ 5 mg/kg group (49.505mg/dL $\pm$ 4.774) ($p < 0.05$).

Conclusion

Intraperitoneal exposure to AFB₁ does not cause significant abnormalities in morphological or biochemical blood tests. No dose-effect relationship.

Keywords: aflatoxin B₁, memory, blood cell count, blood biochemistry.

ARTICLE INFO

PolHypRes 2025 Vol. 91 Issue 2 pp. 73 – 82

ISSN: 1734-7009 eISSN: 2084-0535

DOI: 10.2478/phr-2025-0013

Pages: 11, figures: 1, tables: 1

page **www of the periodical:** www.phr.net.pl

Publisher

Polish Hyperbaric Medicine and Technology Society

Original article

Submission date: 24.02.2025 r.

Acceptance for print: 14.03.2025 r.

INTRODUCTION

Mycotoxins are toxic products of the metabolism of mold fungi common in the environment [1]. Mycotoxins include aflatoxins produced by fungi of the *Aspergillus* genus [2]. Cereal grains are most frequently contaminated with aflatoxins, which results from improper storage [3,4]. Aflatoxins are divided into three groups: B, G and M. Toxins from the first two groups are produced by fungi and occur in the environment, the next group are metabolites of aflatoxins of group B (AFB), present in cow's milk and dairy products. The strongest effects are demonstrated by: AFB1, AFB2 and AFG1, AFG2 [5]. AFB1 metabolism takes place in the liver with the participation of cytochrome P450. Substances are formed that are excreted from the body in urine and bile, as well as adducts that accumulate in the microsomal fraction, combining with nucleic acids and liver proteins. They have mutagenic and carcinogenic effects [6]. The International Agency for Research on Cancer has classified AFB1 as carcinogenic to humans [7]. Aflatoxins have immunotoxic effects [8]. The No Adverse Effect Level-the safe dose of mycotoxins has not been determined [9]. Aflatoxin poisoning can be acute or chronic [10]. Some researchers suggest that aflatoxins influence the development of nervous system degeneration [11]. Abnormalities in cytokine concentrations and disorders in enzymatic systems induced by AFB1 lead to cell dysfunctions. The pro-inflammatory effect of AFB1 can cause immune dysregulation as in neurodegenerative diseases. There are reports confirming it, but further studies are needed [12].

A study on a cell line is not sufficient to determine whether AFB1 will cause disturbances in cytokine concentrations in brain cells in a living organism. Food safety and hygiene are extremely important for the entire human population inhabiting our planet. In times of industrial production of processed food with a long shelf life, it is extremely important that hygiene and safety conditions are ensured at every stage of its production (from field to table), taking into account the well-being of consumers. The State Veterinary Inspection, Sanitary and Epidemiological Stations, the Minister of Agriculture, the Minister of Health in Polish conditions, the bodies of the European Union, the Food and Drug Administration in the USA strive to systematically improve the quality and safety of food. However, we already know that improperly stored (mouldy) food can be a source of danger to people and animals. Mycotoxins present in feed do not decompose in the bodies of farm animals and in the food chain reach the bodies of the final consumers - humans.

THE AIM

The aim of the study was to collect data on the mechanisms of the toxic effects of AFB1 on mammals and to check whether the intraperitoneal route of aflatoxin administration can be used to model poisoning with this mycotoxin in laboratory animals.

MATERIALS AND METHODS

The consent of the Local Ethical Committee was obtained to conduct the experiment (No. 130/2022 of December 19, 2022). The experimental methodology was developed based on available literature [13]. The research was carried out at the Center for Experimental Medicine (OMD) of the Medical University of Lublin in 2024. The experiment was financed by an internal grant DS 755/2024 from the Medical University of Lublin. The experiment used 32 non-pregnant young, healthy female mice of the Albino Swiss strain bred at OMD. The animals weighed approximately 20 g at the start of the experiment. They were randomly divided into 4 groups of 8. The animals were housed in cages of 4. The OMD maintained a 12/12 h light-dark cycle, a constant temperature of 21°C and a constant relative humidity of 55–66%. The first 7 days were devoted to the handling. The mice were allowed to get used to the experimenter by holding them in the hands. The animals had continuous unrestricted access to drinking water and food. The water was sterilized by UV radiation, and the food was provided by Altromin International (Lage, Germany). AFB1 purchased from Sigma Chemical Co. (St. Louis, MO, USA) was used in the experiment. AFB1 was in the form of white crystals. AFB1 was dissolved in physiological saline just before i.p. administration. 0.9% NaCl was purchased from B. Braun, Melsungen AG, Hessen, Germany.

All studies were conducted at fixed hours 8-15. Animals were randomly divided into 4 groups of 8. Animals were administered AFB1 intraperitoneally for 28 consecutive days once a day.

Group I received AFB1 at a dose of 0.1 LD50 (1 mg/kg b.w.).

Group II received AFB1 at a dose of 0.2 LD50 (2 mg/kg b.w.).

Group III received AFB1 at a dose of 0.5 LD50 (5 mg/kg b.w.).

Group IV received 0.9% NaCl.

After the first and last doses (on day1 and day 28), a fresh spatial memory test was performed in the Y-maze. In the test, the mouse was placed inside the Y-maze for 8 min. Spontaneous alternation in the Y-maze is a measure of fresh spatial memory. Each mouse was placed individually in the Y-maze. It consists of 3 compartments of 10 x 10 x 10 cm connected at an angle of 120°. The maze has no floor. A blank sheet of paper was placed under the maze for each mouse to prevent the formation of odor traces. Alternation (defined as consecutive entries into all 3 arms of the maze without repetitions) was assessed. The percentage of alternation was calculated as the ratio of actual possible alternations. The ability to alternate requires that the mice remember which sections had been visited previously. The number of arm entries is also a measure of locomotor activity. The maze used for the study is shown in Figure 1.

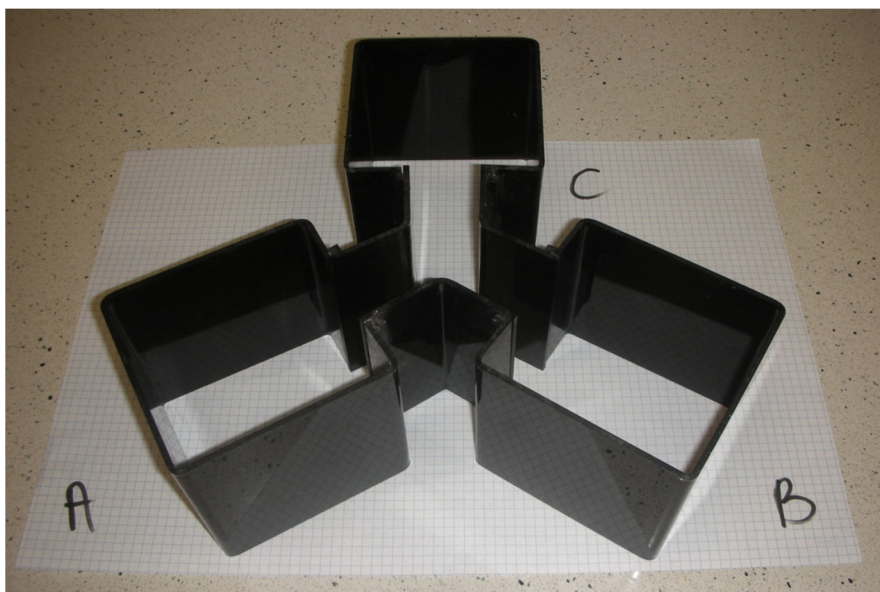


Fig.1 The Y-maze.

After the end of the experiment, the animals were decapitated. No anesthesia was used to prevent possible interactions with blood test results. The blood of the animals was tested in the VetDiagnostyka laboratory in Lublin. To determine the blood cell count, 200 μ l of blood was collected on EDTA. Morphological parameters were measured with an automatic hematology analyzer. The remaining blood was clotted and centrifuged to obtain blood serum. Transaminase activity, bilirubin, cholesterol, triglycerides, gamma-glutamyl transpeptidase, creatinine, urea and protein concentration in blood serum were measured using an automatic biochemical analyzer ErbaMannheim XL-60.

Results are presented as mean $\pm$ standard deviation (SD). Differences between groups were analyzed using analysis of variance, followed by Tukey's post-hoc test. Results were considered statistically significant when $p < 0.05$. Statistical analysis was performed using GrapPad Prism 9 software (Graph Pad Software).

RESULTS

No statistically significant differences were found in the percentage of logical alternations after the first or last dose of AFB1 between the groups of animals. No statistically significant differences were found in the activity of alanine transaminase (ALT), aspartate

transaminase (AST), or gamma-glutamyl transpeptidase (GGTP) between the groups. A statistically significantly lower concentration of total protein was found in the group exposed to AFB1 at a dose of 1 mg/kg body weight compared to the control group ($p < 0.05$). A statistically significant lower concentration of urea was also found after the experiments in the group exposed to AFB1 at a dose of 1 mg/kg body weight compared to the group exposed to AFB1 at a dose of 5 mg/kg body weight ($p < 0.05$). No statistically significant differences were found in the concentration of total bilirubin, total cholesterol, triglycerides in blood serum. Also in the peripheral blood morphology examination no significant differences were found in the number of leukocytes, erythrocytes, thrombocytes and hemoglobin concentration between the groups (Table 1).

The results (mean±SD; n=8).					
	AFB1 1mg/kg	AFB1 2mg/kg	AFB1 5mg/kg	Control	P<0.05
% of spontaneous alternations; day 1 (Y-maze-day1)	55,75±7,46	59,90±6,10	61,89±8,06	63,98±9,81	
% of spontaneous alternations; day 28 (Y-maze-day28)	66,52±11,56	66,66±7,95	64,52±7,50	70,62±7,48	
ALT [U/L]	71,70±26,87	68,75±20,50	80,22±24,33	63,90±14,60	
AST [U/L]	434,25±96,60	436,26±165,67	507,01±338,50	387,32±109,46	
GGTP [U/L]	2,40±0,08	2,75±0,31	2,87±0,39	2,66±0,37	
Protein [g/dL]	4,90±0,24	5,27±0,25	5,36±0,28	5,42±0,27	P<0.05 AFB1 1 mg/kg vs Control
Total bilirubin [mg/dL]	0,07±0,03	0,11±0,05	0,12±0,03	0,09±0,03	
Total cholesterol [mg/dL]	102,92±8,09	110,71±19,61	115,90±31,06	113,93±8,55	
Creatinine [mg/dL]	0,21±0,02	0,21±0,03	0,20±0,02	0,22±0,05	
Urea [mg/dL]	39,05±0,84	44,85±6,00	49,50±4,77	45,93±3,55	P<0,05 AFB11mg/kg vs AFB1 5mg/kg
Triglycerides [mg/dL]	161,62±34,73	202,52±64,62	157,18±27,61	189,78±68,41	
WBC [$10^3/\mu\text{L}$]	9,11±2,02	11,20±2,60	12,53±3,02	8,65±4,22	
RBC [$10^6/\mu\text{L}$]	6,85±1,18	7,15±0,82	7,17±0,80	7,86±0,23	
Hemoglobin (HGB) [g/dL]	15,76±0,37	14,78±1,59	14,30±1,78	15,85±0,43	
Platelets (PLT) [$10^6/\mu\text{L}$]	1127,83±241,07	1189,32±89,81	1287,00±76,89	999,43±241,07	

DISCUSSION

After the first dose of AFB1, animals exposed to this mycotoxin showed a lower percentage of logical alternations in the Y-maze, i.e. they showed poorer spatial memory than animals from the control group, but the differences between groups were not statistically significant and no dose-effect relationship was observed. After 28 days of exposure to AFB1, animals showed slightly better spatial memory than 4 weeks earlier, but the differences between groups were not statistically significant. Exposure to pyrethroid pesticides causes long-term changes in the hippocampus, which results in memory impairment [14]. Gugiandolo et al. showed that the administration of quercetin prevents the neurotoxic effects of AFB1 [15]. However, in their experiment, AFB1 was dissolved in olive oil and administered orally at a dose of 0.75 mg/kg every 3 days for 45 days. Therefore, the dose, solvent, dosing regimen and route of administration were completely different from those in our experiment.

AFB1 crosses the blood-brain barrier. In the study by Yang, it was shown that exposure of mice to AFB1 inhibited the generation of new neurons and increased the proportion of activated microglia in the hippocampus. These neurological changes were

associated with anhedonia, behavioral disorders, and deficits in short-term and social memory [16]. However, the methodology of the study conducted by Yang et al. differed from ours, because they conducted the experiment in male mice that were intragastrically administered aqueous solutions of the xenobiotic.

In the studies of transaminase activity in the groups exposed to AFB1, higher values were found than in the control group, but the differences were not statistically significant. A trend indicating a dose-effect relationship was observed between the AFB1 dose in our experiment and AST activity. The liver enzyme AST has two isoforms: cytoplasmic and mitochondrial. ALT occurs in the cytoplasmic form. Their activity increases in response to damage caused by hypoxia, toxins, drugs, xenobiotics. AST occurs in liver cells, skeletal muscles, cardiac muscle, kidneys, brain and erythrocytes. ALT occurs mainly in liver cells. A physiological increase in aminotransferase activity occurs under the influence of analgesics [17].

In our experiment, in the group exposed to AFB1 at a dose of 1 mg/kg, the concentration of total protein in the blood serum was significantly lower than in the control group. No such differences were observed in the groups exposed to higher doses. Perhaps AFB1 did not dissolve in physiological saline when preparing solutions of 2 mg/kg and 5 mg/kg. Mycotoxin crystals could remain

in the peritoneal cavity undissolved and did not fully affect protein production in the liver. Zhang et al. studied the effect of AFB1 on the integrity of the intestinal barrier of animals. Their experiment consisted of an *in vitro* part, in which porcine jejunal epithelial cells were treated with increasing concentrations of AFB1 (10–60 mg/l) and *in vivo*, in which mice were gavaged with 1% dimethyl sulfoxide (110 mg/kg b.w.) and AFB1 (0.3 mg/kg b.w.) for 28 days. The results of *in vitro* and *in vivo* studies confirmed that exposure to AFB1 has a negative impact on intestinal function and structure [18].

In our experiment, an increasing concentration of total bilirubin in blood serum was observed with exposure to an increasingly higher dose of AFB1, but the differences between groups were not statistically significant. A similar phenomenon was observed in the case of measurements of total cholesterol concentrations in blood serum. There were no statistically significant differences between groups in terms of triglyceride concentrations. Surprisingly, the group of mice exposed to the highest dose of AFB1 had a lower concentration of triglycerides than the other groups. A doubt arose whether AFB1 administered intraperitoneally was absorbed into the blood and reached the liver in full dose. In the studies of renal parameters, a significantly lower ($p < 0.05$) urea concentration was noted in the group exposed to AFB1 at a dose of 1 mg/kg compared to the group exposed to AFB1 at a dose of 5 mg/kg. This

observation also leads us to assume that the higher dose of AFB1 was not absorbed from the peritoneal cavity.

Ingestion is the most common route of exposure to AFB1. After ingestion of a meal contaminated with AFB1, it is metabolized in the liver by a microsomal mixed-function oxidase from the cytochrome P450 family. AFB1 has been shown to cause malnutrition and growth disorders in humans and animals [19]. In experiments on rats administered AFB1, aqueous and vacuolar degeneration, tubular degeneration, epithelial edema and blistering, infiltration of inflammatory cells, and segmental necrosis of the glomeruli were observed in the kidneys [20–29]. Our results are therefore not fully consistent with those of most authors. They most often administered AFB1 dissolved in oil by gavage.

In blood count studies, no significant differences were noted between groups. Imbabi et al. in a study on rabbits, they found that silymarin effectively protected animals exposed to AFB1 in the diet from significant disturbances in blood morphology and biochemical tests of peripheral blood [30].

CONCLUSION

Intraperitoneal exposure to AFB B1 does not cause significant abnormalities in blood morphology or biochemical tests. There is no dose-effect relationship.

REFERENCES

- Lopes P, Sobral MMC, Lopes GR, Martins ZE, Passos CP, Petronilho S, Ferreira IMPLVO. Mycotoxins' Prevalence in Food Industry By-Products: A Systematic Review. *Toxins* (Basel). 2023 Mar 29;15(4):249. doi: 10.3390/toxins15040249. PMID: 37104187; PMCID: PMC10142126.
- Barabasz W, Pikulicka A. Mykotoksyny – zagrożenie dla zdrowia ludzi i zwierząt. Część 1. Mykotoksyny – charakterystyka, występowanie, toksyczność dla organizmów. *J HealthStudy Med*. 2017; 3: 65–108.
- Pleadin J, Frece J, Markov K. Mycotoxins in food and feed. *Adv Food Nutr Res*. 2019;89:297-345. doi: 10.1016/bs.afnr.2019.02.007. Epub 2019 Mar 6. PMID: 31351529.
- Richard J.L.: Some major mycotoxins and their mycotoxicoses- an overview. *Int. J. Food Microbiol.*, 2007; 119: 3-10.
- Lai X., Zhang H., Liu R., Liu C.: Potential for aflatoxin B1 and B2 production by *Aspergillus flavus* strains isolated from rice samples. *Saudi J. Biol. Sci.*, 2015; 22: 176-180.
- Ülger TG, Uçar A, Çakıroğlu FP, Yılmaz S. Genotoxic effects of mycotoxins. *Toxicon*. 2020 Oct 15;185:104-113. doi: 10.1016/j.toxicon.2020.07.004. Epub 2020 Jul 10. PMID: 32653416.
- World Health Organization (WHO), International Agency for Research on Cancer (IARC): Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Lyon, France 56, 1993 <http://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf> (cited on May 10-th2025).
- Meissonnier GM, Pinton P, Laffitte J, et al. Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *ToxicolApplPharmacol*. 2008;231(2):142-149.
- Abia W.A., Warth B., Sulyok M., Krska R., Tchana A., Njobeh P.B., Turner P.C., Kouanfack C., Eyongetah M., Dutton M., Moundipa P.F.: Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. *Food Chem. Toxicol.*, 2013; 62: 927-934.
- Skrzydlewski P, Twarużek M, Grajewski J. Cytotoxicity of Mycotoxins and Their Combinations on Different Cell Lines: A Review. *Toxins* (Basel). 2022 Mar 30;14(4):244. doi: 10.3390/toxins14040244. PMID: 35448853; PMCID: PMC9031280.
- Arce-López B, Alvarez-Erviti L, De Santis B, Izco M, López-Calvo S, Marzo-Sola ME, Debegnach F, Lizarraga E, López de Cerain A, González-Peñas E, Vettorazzi A. Biomonitoring of Mycotoxins in Plasma of Patients with Alzheimer's and Parkinson's Disease. *Toxins* (Basel). 2021 Jul 10;13(7):477. doi: 10.3390/toxins13070477. PMID: 34357949; PMCID: PMC8310068.
- Nguyen VTT, König S, Eggert S, Endres K, Kins S. The role of mycotoxins in neurodegenerative diseases: current state of the art and future perspectives of research. *Biol Chem*. 2021 Aug 27;403(1):3-26. doi: 10.1515/hsz-2021-0214. PMID: 34449171.
- Borzecki, P., Borzecka, A., Chylinska-Wrzes, P., Lis-Sochocka, M., Wawryk-Gawda, E., & Jodłowska-Jedrych, B. (2019). Evaluation of interleukin-6 concentration in the liver of Albino Swiss mice after intoxication with various doses of patulin, *Current Issues in Pharmacy and Medical Sciences*, 32(1), 34-39.
- Nasuti C, Fattoretti P, Carloni M, Fedeli D, Ubaldi M, Ciccocioppo R, Gabbianelli R. Neonatal exposure to permethrin pesticide causes life long fear and spatial learning deficits and Walters hippocampal morphology of synapses. *Journal of Neurodevelopmental Disorders* 2014; 6(1):7-15.
- Gugliandolo E, Peritore AF, D'Amico R, Licata P, Crupi R. Evaluation of Neuroprotective Effects of Quercetin against Aflatoxin B1-Intoxicated Mice. *Animals* (Basel). 2020 May 21;10(5):898. doi: 10.3390/ani10050898. PMID: 32455780; PMCID: PMC7278413.
- Yang C, Jiang W, Su D, Yang C, Yuan Q, Kang C, Xiao C, Wang L, Peng C, Zhou T, Zhang J. Contamination of the traditional medicine *Radix Dipsaci* with aflatoxin B1 impairs hippocampal neurogenesis and cognitive function in a mouse model of osteoporosis. *Ecotoxicol Environ Saf*. 2024 Sep 15;283:116831. doi: 10.1016/j.ecoenv.2024.116831. Epub 2024 Aug 15. PMID: 39151374.
- Mrozikiewicz, P. M.; Bogacz, A. Wpływ Leków Na Wyniki Badań Laboratoryjnych- Wybrane Przykłady Interferencji. *Medycyna po Dyplomie* 2013, 22 (6), 42–48.
- Zhang M, Li Q, Wang J, Sun J, Xiang Y, Jin X. Aflatoxin B1 disrupts the intestinal barrier integrity by reducing junction protein and promoting apoptosis in pigs and mice. *Ecotoxicol Environ Saf*. 2022 Dec 1;247:114250. doi: 10.1016/j.ecoenv.2022.114250. Epub 2022 Nov 2. PMID: 36334341.
- Tchana AN, Moundipa PF, Tchouanguep FM. Aflatoxin contamination in food and body fluids in relation to malnutrition and cancer status in Cameroon. *Int. J. Environ. Res. Public Health*. 2010;7:178–188. doi: 10.3390/ijerph7010178

20. Owumi S, Najophe ES, Farombi EO, Oyelere AK. Gallic acid protects against Aflatoxin B1 -induced oxidative and inflammatory stress damage in rats kidneys and liver. *J. Food Biochem.* 2020;44:e13316. doi: 10.1111/jfbc.13316.
21. Shin KT, Guo J, Niu YJ, Cui XS. The toxic effect of aflatoxin B1 on early porcine embryonic development. *Theriogenology.* 2018;118:157–163. doi: 10.1016/j.theriogenology.2018.06.002.
22. Huang B, et al. Aflatoxin B1 Induces Neurotoxicity through reactive oxygen species generation, DNA damage, apoptosis, and s-phase cell cycle arrest. *Int. J. Mol. Sci.* 2020 doi: 10.3390/ijms21186517
23. Owumi SE, Adedara IA, Akomolafe AP, Farombi EO, Oyelere AK. Gallic acid enhances reproductive function by modulating oxido-inflammatory and apoptosis mediators in rats exposed to aflatoxin-B1. *Exp. Biol. Med. (Maywood)* 2020;245:1016–1028. doi: 10.1177/1535370220936206.
24. Mary VS, Theumer MG, Arias SL, Rubinstein HR. Reactive oxygen species sources and biomolecular oxidative damage induced by aflatoxin B1 and fumonisin B1 in rat spleen mononuclear cells. *Toxicology.* 2012;302:299–307. doi: 10.1016/j.tox.2012.08.012.
25. Adedara IA, Owumi SE, Uwaifo AO, Farombi EO. Aflatoxin B1 and ethanol co-exposure induces hepatic oxidative damage in mice. *Toxicol. Ind. Health.* 2010;26:717–724. doi: 10.1177/0748233710377772.
26. Baptista AS, et al. Utilization of diets amended with yeast and amino acids for the control of aflatoxicosis. *World J. Microbiol. Biotechnol.* 2008;24:2547–2554. doi: 10.1007/s11274-008-9776-5.
27. Colakoglu F, Donmez HH. Effects of aflatoxin on liver and protective effectiveness of esterified glucomannan in merino rams. *Sci. World J.* 2012;2012:462925. doi: 10.1100/2012/462925.
28. Yaman T, Yener Z, Celik I. Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis. *BMC Complement. Altern. Med.* 2016;16:232–232. doi: 10.1186/s12906-016-1217-7.
29. Eraslan G, et al. The effects of diosmin on aflatoxin-induced liver and kidney damage. *Environ. Sci. Pollut. Res.* 2017;24:27931–27941. doi: 10.1007/s11356-017-0232-7.
30. Imbabi TA, El-Sayed AI, El-Habbak MH, Nasr MA, Halawa EH. Ameliorative effects of silymarin on aflatoxin B1 toxicity in weaned rabbits: impact on growth, blood profile, and oxidative stress. *Sci Rep.* 2024 Sep 17;14(1):21666. doi: 10.1038/s41598-024-70623-z. PMID: 39289432; PMCID: PMC11408635.

Barbara Nieradko-Iwanicka

Zakład Higieny i Epidemiologii Uniwersytet Medyczny w Lublinie
ul Chodźki 7, 20-093 Lublin
e-mail: barbara.nieradko-iwanicka@umlub.pl