Effect of Aflatoxin B1 Contaminated Corn and Their Products on Some Physiology Parameters in Laboratory Rats

Mustafa Q. Khaled and Karkaz M. Thalij

1,2Department of Food Science, College of Agriculture, University of Tikrit, Iraq.

Email: kthalij@tu.edu.iq

Abstract

This study was conducted in the laboratories of the Department of Food Sciences/College of Agriculture and in the animal house of the College of Veterinary Medicine and the Central Laboratory at Tikrit University for the period from the beginning of September 2020 to the end of February 2021, with the aim of identifying the types of fungi contaminating corn grains and their products in Iraqi stores and markets and estimating the concentration of Aflatoxin B1 toxins using ELISA technique, the results of Aflatoxin B1 tests showed that all samples contained a higher percentage of what is allowed to be used in human food, which was between (39.5-29) µg/g. The results also showed that feeding of corn samples and its products contaminated with Aflatoxin B1 to rats fed for 21 days affected the significant decrease (p<0.05) in body weight and weight gained for laboratory rats, as well as liver, kidney and spleen enlargement for those animals. There was also a significant decrease in the number of red blood cells and hemoglobin and an increase in the number of white blood cells compared with the control group. The results also showed a significant increase in the activity of liver enzymes for AST, ALT and ALP, and an increase in the concentration of renal parameters for both urea and creatinine concentrations.

Keywords: Corn, Aflatoxins B1, ELISA, Blood

1.Introduction

Corn (Zea mays) is a herbaceous plant belonging to the family Poaceae. It is considered one of the most important cereal crops, as it is grown in large parts of the world due to its great adaptability to various environments and it is the main source of human and animal food [1]. Corn oil is the main oil that is marketed as vegetable, as it is obtained from corn seed and is used in the biodiesel, soap, paints and coating industries [2]. Corn is an important source of minerals, dietary fiber and vitamins, as well as containing carbohydrates in large quantities. Therefore, it is used as food in fresh form or as an important ingredient in many dishes in many countries of the world [3]. Corn can be contaminated with fungi and infection depends on its biological composition and environmental physiological conditions. Some of these fungi produce toxins when infecting corn that become toxic to humans and pet species when ingested in large quantities and these are called mycotoxins [4].

The most important mycotoxins that affect cereals, food and forage are Aflatoxins, whose name is derived from the type of fungus Aspergillus flavus, which Aspergillus parasiticus can also produce. These toxins are produced from the types of fungi that produce it in various types of foods, especially cereals, nuts, dairy products, tea, spices and cocoa, in addition to animal and fish feed [5]. Exposure to foods contaminated with high levels of Aflatoxin can lead to immediate death in humans and animals. High levels of the toxin worsen health through liver damage and immunosuppression [6]. As for its other effects, it was found that it causes stunting in children when ingested through breast milk or foodstuffs given to the child [7]. The harmful effect of Aflatoxins in humans ranges from acute hepatotoxicity to chronic diseases such as liver cancer, hemorrhage, edema and even immediate death. It has also been reported that prolonged consumption of Aflatoxins causes impaired immune function, malnutrition, stunted child growth, and a number of disabilities and death [8].

2.Materials and Methods

2.1.Collect samples

The food samples for yellow corn and its products included 12 brands representing more than one different local and imported origin, with 6 replicates for each brand. It was one of the types offered in the Iraqi markets for the city of Tikrit and Erbil and one of the types desired by consumers. The conformity of the storage conditions and shelf life for human
consumption, as well as the external appearance and definition of the contents and the cleanliness of the samples as mentioned in [9].

2.2. Determination of Aflatoxin B1 toxin concentration in maize samples and its products

The concentration of Aflatoxin B1 was estimated using an ELISA device (Bio-tek Company) using a test kit for the determination of afla B1 supplied by Shenzhen Lvshiyuan Company (Chinese). It was determined by weighing (10) g of each sample of maize or its products, and then grinding it using a mill Electricity, taking into account the cleaning and sterilization of the mill after each meal, grinding for each of the samples. Weight (1) g of each sample and put it in a (50) ml tube designated for centrifugation and add to it (5) ml of the previously prepared extraction solution according to what was mentioned in the test kit manual, then a centrifugation was performed. For samples at a speed of (4000) rpm for (10) minutes at a temperature of (18). Take (100) μl of the sample yoghurt and add to it (700) μl of the re-dissolving solution supplied with the test kit and leave it for (10) minutes with continuous stirring, then take (100) μl of it for the examination and the calculations were made according to What is stated in the manufacturer's manual for the scan kit.

2.3. Total Red Blood Cell count (RBCs)

For the purpose of counting red blood cells, the blood sample was diluted at a ratio (1:200) using Hymes'solution. After mixing the blood well with the dilution solution, a drop of the cell suspension was placed in the counting chamber designated for this purpose. After fixing the glass cover on the counting slide, it was left for (1-2) minutes for the erythrocytes to stabilize, the number of red blood cells (n) was calculated in five medium squares, and then the total number was extracted, depending on what mentioned [10].

2.4. Total White Blood Cell count (WBCs)

The measurement of the total number of white blood cells was adopted in the manner indicated by [11], as blood was withdrawn by means of a special pipette to count white blood cells. The blood was diluted using Turky's solution, then the blood was mixed with the diluted mixture well and a drop was placed. From it on the enumeration slide after leaving the first drops and leaving the slide for two minutes to settle the pellets, then count the number of white blood corpuscles in the central square of the enumeration slide under the power of magnification (40X).

2.5. Estimation of Hemoglobin Concentration (Hb)

The hemoglobin concentration was estimated by taking a test tube containing 5 ml of Drabkin's solution and adding (0.2) ml of blood to it, then the contents were mixed well using the rotary mixer and left for (10) minutes, then the transmittance was measured using a spectrophotometer at a wavelength of (540) nm after the device was cleared With Drabkin's solution, the amount of hemoglobin was calculated using the standard curve representing the relationship between permeability and the amount of hemoglobin per (100) ml [12].

2.6. Determination of the activity of Aspartate Amino Transferase (AST) in blood serum

The enzymatic method was used to estimate the activity of the AST enzyme in the blood serum. The method included the use of a kit assay supplied by Randox Company (British), and the method is based on the ability of the enzyme to act on the base substance (aspartic acid and alpha-ketoglutaric acid) and convert the amino acid aspartic acid to alpha Ketone (pyruvic), which can be converted into a reddish brown derivative compound by adding the reagent (2.4) Di-Nitrophenyl hydrazine, and the absorbance is read using a spectrophotometer at a wavelength of (505) nm.

2.7. Determination of the activity of Alanine Amino Transferase (ALT) in blood serum

The enzymatic method was used, as it used the analysis kit (Kit) supplied by Randox Company (British), and the ALT enzyme works on the base material (alanine and alpha-ketoglutaric acid) by converting the amino acid alanine to pyruvic acid directly, which easily turns into a compound derived from hydrazine with a brown color Reddish brown by adding the reagent (2.4) Di-Nitrophenyl hydrazine, and the absorbance is read on a spectrophotometer at a wavelength of (505) nm depending on [13].
2.8. Determination of Alkaline Phosphatas (ALP) activity in blood serum

As far as the effectiveness of (ALP), it was done by using standard crews equipped with the company ROCHE (Germany) and the results were read using the Swiss-origin Reflotron device according to the instructions of the supplying companies as mentioned in [14].

2.9. Determination of Urea in blood serum

The blood urea concentration was estimated by standard crews equipped with Linear Chemicals (Spain), and the analyzes were also carried out by a spectrophotometer according to the instructions of the processing companies as mentioned in [15].

2.10. Determination of Creatinine blood serum

Custom test kits of BIOLABO (France) were used, then reading the absorbance of the models at a wavelength of 490 nm and calculating creatinine in the blood serum using the following equation [16].

3. Results and Discussion

3.1. The concentration of Aflatoxin B1 in food samples

Table (1) shows the results of estimating the concentration of Aflatoxin B1 in samples of maize and its products collected from the Iraqi markets in the city of Tikrit. The results showed that the samples of maize and its products examined, whether local or imported, contained concentrations of aflatoxin B1 between 20.6 to 39.5 µg/kg. The results agreed with [17], who found that maize grains in Baghdad city and some Iraqi governorates were contaminated with aflatoxin B1 at concentrations between 270 to 500 µg/kg. The results also agreed with [18], who found that maize kernels and their products were contaminated with Aflatoxin B1 in Sri Lanka with a range of 60 to 70 µg/kg. The reason for the presence of aflatoxin in the samples could be from its production in the field after being infected with the fungus A.flavus in the field, in addition to its infection in the stores [19]. The change of environmental conditions such as temperature and humidity when corn is growing in the field or when it is stored in stores can be considered as the main cause of infection with toxin-producing fungi of A. flavus and A. parasiticus, as it caused the employment of their metabolic processes in the production of these Toxins [20]. The US Food and Drug Administration (FDA) has determined the permissible levels of aflatoxin B1 in foods intended for direct human consumption in concentrations not to exceed 20 μg/kg, as well as not exceeding the same concentration in animal feed and feed ingredients intended for dairy and poultry products. We can conclude that most samples of corn and its products are ineligible for human consumption, except for one sample, which is imported canned corn, whose percentage was close to the percentage allowed by the US Food and Drug Administration (FDA), because the concentration of Aflatoxin B1 in these samples has exceeded the permissible limit.

Table 1. Concentration of Aflatoxin B1 in samples of corn and its products.

| No. | Sample Name                | Concentration AFB1/µg/kg |
|-----|----------------------------|-------------------------|
| 1   | Ben AL-Nahrain Store Corn  | 24.2                    |
| 2   | Dalya Chips                | 32.7                    |
| 3   | Imported Corn flakes      | 27.1                    |
| 4   | Boshar Chips               | 24.2                    |
| 5   | Imported Corn Sacks       | 29.6                    |
| 6   | Corn Nuts                 | 29.2                    |
| 7   | Imported Cheetos Chips    | 39.5                    |
| 8   | Local Corn Sacks          | 27.3                    |
| 9   | Local Kernel Corn         | 37.9                    |
| 10  | Local Canned Corn         | 37.2                    |
| 11  | Local Market Corn         | 21.9                    |
| 12  | Imported Canned Corn      | 20.6                    |

3.2. Effect on Blood Picture Parameters

Table (2) shows. Effect of feeding status on maize samples or their products contaminated with different concentrations of Aflatoxin B1 on blood picture parameters of laboratory rats fed them for 21 days. The results showed that the blood picture parameters for both hemoglobin and total numbers of red blood cells decreased significantly (p<0.05) in the group of T2 rats fed a diet containing Aflatoxin B1 and their values were at 9.433 and 5.520, respectively, compared to their values in the
control group that they were 10.532 mg/L and 6.532 (×10^6/m³), respectively, while no significant differences were recorded between the rest of the groups, but also decreased compared with the control group for the numbers of red blood cells and the level of hemoglobin, and the values of the groups T3, T4 and T5 were at 5.225, 5.355, 5.312 (×10^6/m³) and 9.375, 9.405, and 9.320 (mg/L), respectively, compared with their values for each of the numbers of red blood cells and hemoglobin that were in T1 control group animals at 6.532 and 10.532 (mg/L).

The results also showed that the total numbers of white blood cells increased significantly (p<0.05) in the animals fed with aflatoxin B1 in all their concentrations, and it was in the T2 group at 12.875 compared with their numbers in the blood of the animals of the control group, which was at 10.050 (×10^6/m³). The obtained results agreed with Ramamurthy and Rajakumar, (2016) who found that feeding laboratory animals with aflatoxin in India led to a decrease in the number of red blood cells (RBCs) and hemoglobin (Hb) and an increase in the number of white blood cells (WBCs) compared with their values in control group animals.

**Table 2.** Blood picture parameters for rats fed samples of maize and its products contaminated with different levels of aflatoxin B1.

| Treatments | WBCs (×10^6/m³) | RBCs (×10^6/m³) | Hb (mg/L) |
|------------|-----------------|-----------------|-----------|
| T1         | 10.050 ±0.53 b  | 6.532 ±0.23 a   | 10.532 ±0.20 a |
| T2         | 12.175 ±0.46 a  | 5.520 ±0.20 b   | 9.433 ±0.10 b  |
| T3         | 11.450 ±0.30 ab | 5.225 ±0.12 ab  | 9.375 ±0.15 ab |
| T4         | 11.900 ±0.67 a  | 5.355 ±0.26 ab  | 9.405 ±0.22 ab |
| T5         | 11.850 ±0.51 a  | 5.312 ±0.22 ab  | 9.320 ±0.20 ab |

*Different letters within the same column indicate a significant difference at the 0.05 probability level.

The reason for the decrease in the level of the total number of red blood cells in the rats treated with aflatoxin is attributed to the effectiveness of the toxins in causing anemia, which may include a down-regulation of the activity of erythropoietin, which contributes to the decrease in the level of (Transient erythroblastopenia of Childhood) TEC, causing Reducing the effectiveness of the toxins in causing anemia, which may include a down-regulation of the activity of erythropoietin, which contributes to the decrease in the level of (Transient erythroblastopenia of Childhood) TEC, causing Reducing the effectiveness of the toxins in causing anemia, which may include a down-regulation of the activity of erythropoietin, which contributes to the decrease in the level of (Transient erythroblastopenia of Childhood) TEC, causing

3.3. Effect on Liver Enzymes

The effect of feeding status on samples of maize or its products contaminated with different concentrations of aflatoxin B1 on the activity of liver enzymes in laboratory rats fed on them for 21 days is shown in Table (3). The results showed that aflatoxins, when fed on them in contaminated samples of maize or their products, caused a significant (p<0.05) increase in the activity of liver enzymes for AST, ALT and ALP in treatments T2, T3, and T4. 49.75, 46.25 and 48.75 mg/L for AST and at 45.25, 40.25, 44 mg/L for ALT and 69.01, 52.31, 57.24 mg/L for ALP, respectively, compared with its values in the control group animals were 44.75 mg/L in the case of AST enzyme, 36.75 mg/L in the case of ALT enzyme, and 49.41 mg/L in the case of ALP enzyme.

**Table 3.** The activity of liver enzymes of rats fed on samples of maize and its products contaminated with different levels of aflatoxin B1.

| Treatments | ALP (Mg/L) | ALT/GPT (Mg/L) | AST/GOT (Mg/L) |
|------------|------------|----------------|----------------|
| T1         | 49.41 ±1.11 b | 36.75 ±3.03 b | 44.75 ±1.43 b |
| T2         | 69.01 ±1.79 a | 45.25 ±1.11 a | 49.75 ±1.88 a |
| T3         | 52.31 ±1.76 ab| 40.25 ±1.65 ab| 46.25 ±1.75 ab|
| T4         | 57.24 ±1.91 ab| 43.00 ±1.12 ab| 48.75 ±1.96 a |
| T5         | 50.91 ±1.88 b | 37.00 ±1.19 b | 45.50 ±1.93 b |

*Different letters within the same column indicate a significant difference at the 0.05 probability level.

T1: control and T2: AFB1 at a concentration of 39.5 μg/kg, T3: AFB1 at a concentration of 32.7 μg/kg, T4: AFB1 at a concentration of 37.9 μg/kg and for T5: AFB1 at a concentration of 29.0 μg/kg.
The obtained results agreed with [22], who indicated that rats fed aflatoxin in Germany had an increase in AST, ALT and ALP enzyme activities. The results also agreed with [23], in China, who showed that chicken chicks fed on Corn contaminated with aflatoxins had an increase in enzyme activity.

The reason for the increase in the activity of liver enzymes can be due to the exposure of the liver to aflatoxin B1, which causes damage to hepatocytes and an increase in the permeability of their membranes, which causes the enzymes in the liver to be released into the bloodstream and thus increase their concentration in the blood serum [24].

### 3.4. Effect on Kidney Function

Table (4) shows the effect of feeding status on maize samples or their products contaminated with different concentrations of aflatoxin B1 on kidney function in laboratory rats fed on them for 21 days. The results obtained showed that the effect of different concentrations of aflatoxin B1 on the level of creatinine and urea in the blood serum of rats fed aflatoxin B1 for each of groups T2, T3, T4 and T5 increased significantly (p<0.05) for both creatinine and Urea and their values of urea were at 46.50, 47.50, 43.50 and 42.25 mg/ml, respectively. As for creatinine values, they were at 0.42, 0.33, 0.37, and 0.31 mg/ml compared with their values in control group animals, which were 39.75 and 0.29 mg/ml, respectively.

The obtained results are in agreement with [25], who indicated that feeding aflatoxin B1 to rats at a concentration of 75 μg/kg for 28 days resulted in an increase in urea from 3.49 to 7.05 mg/ml and creatinine from 16.92 to 34.37 mg/ml.

**Table 4.** Kidney functions of rats fed samples of maize and its products contaminated with different levels of aflatoxin B1.

| Adjectives | Creatinine (mg/ml) | Urea (mg/ml) |
|------------|--------------------|--------------|
| T1         | 0.29 ±0.55 b       | 39.75 ±1.79 b|
| T2         | 0.42 ±0.13 a       | 46.50 ±1.32 a|
| T3         | 0.33 ±0.16 ab      | 47.50 ±1.55 a|
| T4         | 0.37 ±0.12 a       | 43.50 ±1.10 ab|
| T5         | 0.31 ±0.35 ab      | 42.25 ±1.65 ab|

*Different letters within the same column indicate a significant difference at the 0.05 probability level.

T1: control and T2: AFB1 at a concentration of 39.5 μg/kg, T3: AFB1 at a concentration of 32.7 μg/kg, T4: AFB1 at a concentration of 37.9 μg/kg and for T5: AFB1 at a concentration of 29.0 μg/kg.

The effect of aflatoxin B1 on kidney function could be one of its negative effects on kidney cells and causing damage, which caused a decrease in their ability to filter, which caused the excretion of creatinine and urea in a greater amount than normal levels into the blood [21]. The toxic effects of AFB1 on renal function could be in the increase in plasma creatinine concentrations through an increase in its secretion from the muscle or a decrease in its excretion from the kidneys in general [16].

### Conclusion

Aflatoxins B1 were found with concentrations between 20.7 to 39.5 μg/kg of corn grain and its products, and they caused a decrease in hemoglobin and red blood cells, an increase in the number of white blood cells, an increase in the concentration of liver enzymes AST, ALT and ALP, and an increase in urea and creatinine.

### References

[1] Abbas, H. K., Shier, W. T., Plasencia, J., Weaver, M. A., Bellaloui, N., Kotowicz, J. K., ... & Zablotowicz, R. M. (2017). Mycotoxin contamination in corn smut (Ustilago maydis) galls in the field and in the commercial food products. Food Control, 71, 57-63.

[2] Alam, M., N.M. Alandis. (2014). Corn oil based poly (ether amide urethane) coating material Synthesis, characterization and coating properties. Industrial Crops and Products. 57: 17-28.

[3] Alassi, B. Shahad and Ahmed A. Allaw.2020. Effect of adding of the Milk Thistle (SILYBUM MARIANUM) seed powder in the traits of biochemical blood of the quail. Plant Archives Vol. 20, No. 1, pp. 962-964.

[4] APHA (American Public Health Association).(1998). Standard methods for examination of water and wastewater. 20th ed. N.Y.

[5] Arafat, R. Y., & Khan, S. H. (2017). Evaluation of humic acid as an aflatoxin binder in broiler chickens. Annals of animal science, 17(1), 241.

[6] Bregman, B. S. (1987). Spinal cord transplants permit the growth of serotonergic axons across the site of neonatal spinal cord transection. Developmental Brain Research, 34(2), 265-279.

[7] Chu, X., Wang, W., Ni, X., Li, C., & Li, Y. (2020). Classifying maize kernels naturally infected by fungi using near-infrared hyperspectral imaging. Infrared Physics & Technology, 105, 103242.

[8] Eraslan, G., Sarica, Z. S., Bayram, L. Ç., Tekeli, M. Y., Kanbur, M., & Karabacak, M. (2017). The effects of diosmin on aflatoxin-induced liver and kidney damage. Environmental Science and Pollution Research, 24(36), 27931-27941.
[9] Food and Agriculture Organization (FAO). (2000). Aflatoxin research on grain in Asia – its problems and possible solutions. FAO Technical Report.

[10] Hassan, F. F., Al-Jibouri, M. H., & Hashim, A. K. J. (2014). Isolation and Identification of Fungal Propagation in Stored Maize and detection of aflatoxin B1 Using TLC and ELISA Technique. Iraqi Journal of Science, 55(2B), 634-642.

[11] Jayaratne, W. M. S. C., Abeyratne, A. H. M. A. K., De Zoysa, H. K. S., Dissanayake, D. M. R. B. N., Bamunuarachchige, T. C., Waisundara, V. Y., & Chang, S. (2020). Detection and quantification of Aflatoxin B1 in corn and corn-grown soils in the district of Anuradhapura, Sri Lanka. Hellyon, 6(10), e05319.

[12] John, V. D. and Lewis, S. M. (1984). Basic hematological techniques, Practical Hematology; 6th (ed) Pp: 22-45.

[13] Kogbe, J. O. S., & Adediran, J. A. (2003). Influence of nitrogen, phosphorus and potassium application on the yield of maize in the savanna zone of Nigeria. African journal of biotechnology, 2(10), 345-349.

[14] Liu, J. B., Yan, H. L., Cao, S. C., Hu, Y. D., & Zhang, H. F. (2020). Effects of absorbents on growth performance, blood profiles and liver gene expression in broilers fed diets naturally contaminated with aflatoxin. Asian-Australasian journal of animal sciences, 33(2), 294.

[15] Moreau, R. A., Hicks, K. B., Johnston, D. B., & Laun, N. P. (2010). The composition of crude corn oil recovered after fermentation via centrifugation from a commercial dry grind ethanol process. Journal of the American Oil Chemists’ Society, 87(8), 895-902.

[16] Owumi, S., Najophe, E. S., Farombi, E. O., & Oyelere, A. K. (2020). Gallic acid protects against Aflatoxin B1-induced oxidative and inflammatory stress damage in rats kidneys and liver. Journal of Food Biochemistry, 44(8), e13316.

[17] Pankaj, S. K., Shi, H., & Keener, K. M. (2018). A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends in Food Science & Technology, 71, 73-83.

[18] Ramanurthy, V., & Rajakumar, R. (2016). Studies on Ethanolic Leaf Extract of Phyllanthus Niruri and Its EFFECT on Aflatoxin Intoxicated Male Albino Rats. International Journal of Zoology and Applied Biosciences, 1(1), 1-6.

[19] Rodrigues, I. and Chin, L. J. (2012). A comprehensive survey on the occurrence of mycotoxins in maize dried distillers’ grain and solubles sourced worldwide. J. Wor. Myco. 5: 83–88.

[20] Rotimi, O. A., Rotimi, S. O., Oluwafemi, F., Ademuyiwa, O., & Balogun, E. A. (2016). Coexistence of aflatoxicosis with protein malnutrition worsens hepatic oxidative damage in rats. Journal of biochemical and molecular toxicology, 30(6), 269-276.

[21] Schottelius, B. A.; Thomson, J. D. and Schottelius, D. D. (1988) Physiolgy laboratory manual 4th; Mosby company; Saint Louis.science and technology, 52(6): 3756-3762.

[22] Titez , N. W. (2005). Fundamental of Clinical Chemistry, 3 th ed. Saunders, 478- 259.

[23] Waliyar, F., Siambi, M., Jones, R., Reddy, S. V., Chibonga, D., Kumar, P. L., & Denloye, S. (2008). Institutionalizing mycotoxin testing in Africa. Detection Methods, Management, Public Health and Agricultural Trade, 359.

[24] Wang, D., Lindemann, M. D., & Estienne, M. J. (2020). Effect of Folic Acid Supplementation and Dietary Protein Level on Growth Performance, Serum Chemistry and Immune Response in Weanling Piglets Fed Differing Concentrations of Aflatoxin. Toxins, 12(10), 651.

[25] Yilmaz, S., Kaya, E., Karaca, A., & Karatas, O. (2018). Aflatoxin B1 induced renal and cardiac damage in rats: Protective effect of lycopene. Research in veterinary science, 119, 268-275.