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INFLUENCE OF OKRA EXTRACT SUPPLEMENTATION ON SOME HAEMATOLOGICAL PARAMETERS OF MALE MICE EXPOSED TO AFLATOXIN

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ABSTRACT

This research was directed to determine the influence of an alcoholic extract of okra on the lessening of the destructive impact of the aflatoxin produced by Aspergillus fumigatus in white mice and its influence on some physiological blood parameters. Different food samples, (grains and fruits) such as (wheat, barley, corn, rice, citrus, strawberries, and apples) were selected for the isolation of a variety of fungi. The results showed that Aspergillus flavus 15(18.7%), Aspergillus niger12(15%), Penicillium spp 7(8.7%), Aspergillus terreus 7 (8.7%), Aspergillus fumigatus7(8.7%), Alternaria spp. 10 (12.5%), Aspergillus parasiticus 6 (7.5%) Fusarium 6 (7.5%), Penicillium chrysogenum5(6.3), Mucor spp.2(2.5%), and Rhizopus stoloinfier 3(5.5%). The identified fungi were tested for aflatoxin production, and the results revealed that Aspergillus fumigatus produced the most aflatoxin. Okra alcoholic extract was tested in vivo against the negative impact of aflatoxins using different concentrations. The findings revealed that alcoholic extracts showed reasonable influence on some blood parameters, and the results are promising.

INTRODUCTION

Toxications caused by mycotoxins are one of the most serious issues that arise as a result of unconditioned food and food storage. Mycotoxins are fungi-produced toxic metabolic byproducts (Dönmez, 2017). Mycotoxins are naturally produced by fungi on crops in the field, during harvest, and during storage (AS et al., 2018). Mycotoxicosis can occur as a result of consuming contaminated food. The most dangerous mycotoxin is aflatoxin, which is the most prevalent of these mycotoxins. At temperatures above 27°C (80°F), humidity levels above 62%, and feed moisture levels above 14%, aflatoxins are more likely to be produced in feeds (Jiang 2021). Aflatoxin-contaminated food consumption by humans and animals results in serious health issues

and significant financial losses. Aflatoxisis can affect any animal species, but it most frequently affects chickens, sheep, and cattle (Womack et al.,2014). Aflatoxicosis causes several imperfections in organs and tissues, including a reduction in growth rate, an increase in death rate, immunosuppression, anemia, and an increase in coagulation time; it also deteriorates lipid, carbohydrate, and protein metabolism (Womack et al.,2014). Aflatoxicosis impairs lipid, carbohydrate, and protein metabolism and results in a number of organ and tissue defects, as well as a slowing of growth, an increase in mortality, immunosuppression, anemia, and an increase in the time it takes for blood to clot. Okra (*Abelmoschus esculentus L.*) is a common vegetable crop with good nutritional significance and some therapeutic benefits, making it a potential candidate for the use of a variety of nutraceuticals (Elkhalifa *et al.* 2021). The okra fruit's medicinal properties are attributed to the presence of certain significant bioactive components in the mucilage, seed, and pods of the fruit. Aflatoxin's toxic effects have been reported to cause significant changes in biochemical and hematological parameters. Prior to the onset of clinical symptoms, biochemical and hematological parameters change in cases of chronic and subclinical aflatoxicosis (Wang. *et al* 2019). In cases of aflatoxicosis, there are significant alterations in serum biochemical and hematological parameters, which can help with the identification of toxicities Hojnik, *et al*., 2017). There is a high demand for efficient decontamination technology as the removal of AF from contaminated food and foodstuffs continues to be a major problem. Through physical, chemical, or biological means, decontamination procedures have primarily focused on destroying, inactivating, or removing AF (Grover and Joshi., 2014) . There has been a recent paradigm shift away from conventional therapies and toward relatively safer phytotherapies. For the management of various chronic diseases, this divergence is essential. Okra *(Abelmoschus esculentus L.)* is a common vegetable crop that has therapeutic benefits in addition to good nutritional significance, making it a potential candidate for use in a variety of functional ingredients. It has been demonstrated that using biological or chemical agents in animal feeds can reduce the negative effects of aflatoxin. Okra extracts either partially or completely counteracted the impact of AF on performance, biochemistry, hematology, and immune response, according to studies using it at various concentrations (Husen, et al., 2019; Chen et al., 2005; Devanesan et al., 2021). This study is the first of its kind to investigate the effect of adding okra extract to the diet of an aflatoxicosis animal to see how it affected some hematological parameters.

METHODOLOGY

Isolation of fungi from Grain and fruits materials

The grains and fruits were superficially disinfected by soaking them for one minute in a 1% aqueous sodium hypochlorite solution, followed by three separate rinses in sterile distilled water. According to (Sultan at al., 2022). the grains were blotted dry between sterile Whatman No. 1 filter paper and then plated on Potato Dextrose Agar (PDA). The plates were then incubated at a temperature of 25 °C for 7 days. To create axenic cultures, mixed growth was subsequently subculture.

Identification of a fungal isolate by morphology

According to colony morphology and microscopic examination, the isolated fungi were identified as described in (Marcos and Pincus 2012).

Detecting the ability of isolates to produce aflatoxin

Method (Kumar et al., 2007) was employed to detect the ability of fungal isolates to produce aflatoxins (which were inoculated by the stabbing method from a 5-day-old culture). After five

days of incubation at 25°C, the growth plate cover's internal surface was covered with filter paper that had been soaked in a 25% ammonia solution. After 24 hours of upside-down incubation at 25°C, the results were documented.

Plant Identification

Taxonomic identification of Okra (*Abelmoschus esculentus*) fruit was carried out by the Department of Food Sciences, Faculty of Agriculture, University of Kufa, Najaf, Iraq.

Plant Material and Extraction

The okra fruit weighed about 2 kilograms and was obtained from the market in Najaf, Iraq. After removing the pods and chopping the fruit into 2 mm pieces, it was dried for 3-5 days before being ground into powder. A bottle containing 500 mg of the powder was filled with 1.5 L of 96% ethanol, which was then softened. The bottle was then shaken 100 times per day for three days, continuously, until the solvent was clear. A crude extract was produced by evaporating the solvent using the Rotavapor® R-300 (Buchi) at a temperature of about 50 °C. Next, a freeze dryer was used to dry the crude extract.

Experimental Animals and Ethical Clearance

This study used healthy adult male mice (Mus musculus), strain BALB/C, with an age range of 3–4 months and a body weight range of 30–40 g, obtained from the Faculty of Science, University of Kufa, Najaf, Iraq. Mice were acclimatized for 2 weeks to provide conditions that were similar to those in the animal laboratory at the Faculty of Science University of Kufa, Najaf, Iraq. All body weight and blood levels were recorded before and after the administration of afaltoxin. Mice were divided into 3 groups (n = 5 mice) details below). Mice were in control of environmental conditions (25±5 °C, humidity of 50±10% and 12 light/dark cycles).

The mice were fed as follows and continuously given clean water throughout the eight-week experiment.

Group 1 (C) was fed a mouse-specific diet (control).

Group 2 (A) was fed food containing 250 µg/day of aflatoxin per day.

Group 4 (B) was fed food containing 250 µg /day of aflatoxin and 250 µg / day okra extract.

Mice were fed standard pellets and water (ad libitum). All treatment procedures have been tested through Ethical Clearance at Kufa University (Approval Reference Number: 2.KE.069.04.2023).

Blood Collection and analysis

Blood samples were collected at the conclusion of the experiment and placed in tubes with anticoagulant added. The values of the hematocrit, hemoglobin, white blood cells (WBC), red blood cells (RBC), differential leukocyte counts, and hematocrit values were calculated. Haemoglobin amounts were determined using a commercial kit (Biosystem), and the red blood cell (RBC), PVC, and white blood cell (WBC) counts were determined using a haematocytometer.

RESULTS AND DISCUSSION

A total of 16 isolates were revealed through the study from different food sources (grains and fruits). The samples obtained from different local markets in Najaf. The result showed the isolation and identification of ten fungal genera from seventy different samples of grain (wheat, barley, corn, and rice), with occurrences of *Aspergillus flavus* 15(18.7%), *Aspergillus niger12(15%), Penicillium spp 7(8.7%), Aspergillus terreus 7 (8.7%), Aspergillus fumigatus7(8.7%), Alternaria spp. 10 (12.5%), Aspergillus parasiticus 6 (7.5%) Fusarium 6 (7.5%), Penicillium chrysogenum 5(6.3), Mucor spp. 2(2.5%) and Rhizopus stoloinfier 3(5.5%),* while six fungal genera were identified from 25 fruit samples (citrus, strawberries, and apples), with prevalence rates shown in table 2. The most common genera isolated were *Aspergillus* spp. and *Penicillium* spp. Among *Aspergillus* species, the strains identified were *A. flavus*, which is known to produce aflatoxins, and *A. niger*, which is known to produce ochratoxin A.

Table-1: Frequency of occurrence of fungal species with selected grain

Fungi isolates	Grain infected	Number of Fungi isolates	Frequency of occurrence (%)
Aspergillus flavus	Wheat,barley,corn,rice	15	18.7%
Aspergillus niger	Wheat,barley,corn,rice	12	15%
Aspergillus terreus	Wheat,barley,corn,rice	7	8.7%
Aspergillus fumigatus	Wheat,barley,corn,rice	7	8.7%
Alternaria spp.	Barley,corn,rice	7	8.7%
Aspergillus parasiticus	Wheat,barley, ,rice	6	7.5%
Fusarium	Wheat,barley,corn,	6	7.5%
Penicillium chrysogenum	Wheat, ,corn,rice	5	6.3%
Mucor spp.	Barley,corn,rice	2	2.5%
Rhizopus stolonifer	Wheat,barley	3	5.5%

Table-2: Frequency of occurrence of fungal species with spoilt fruits

Fungi isolates	Fruit infected	Number of Fungi isolates	Frequency of occurrence (%)
Penicillium spp.	Citrus,Strawberry,apples	10	12.5%
Aspergillus flavus	Citrus,Strawberry,apples	7	8.7%
Aspergillus niger	Citrus,Strawberry	2	2.5%
Aspergillus fumigatus	Strawberry,apples	2	2.5%
Aspergillus terreus	Citrus,Strawberry,apples	2	2.5%
Aspergillus parasiticus	Citrus,Strawberry	2	2.5%

Table 3 shows the ability of fungus isolates to produce aflatoxin using the ammonia hydroxide method. The results revealed that the highest-level fungal isolate producing aflatoxin is *Aspergillus fumigatus.* Therefore, aflatoxin was collected from this isolate and further processed along with okra extract for the assessment of some blood parameters.

Table -3. Fungal isolates producing aflatoxin using the ammonia hydroxide method

Fungal isolates	Aflatoxin production
Aspergillus flavus	+
Aspergillus niger	+
Aspergillus terreus	+
Aspergillus fumigatus	+
Alternaria spp.	+
Aspergillus parasiticus	+
Fusarium	_
Penicillium chrysogenum	_
Mucor spp.	_
Rhizopus stolonifer	_

Aflatoxin production Detected = "+", Not detected = "- ".
The results in table 4 revealed effects of aflatoxin on some haematological parameters (n = 4).
Table 4: Effects of AF on some haematological parameters (*n* = 4).

Parameters	Fed with the normal diet for mice(control)	Fed with the food containing 150 *u*g/day aflatoxin .	Fed with the food containing 200 *u*g /day aflatoxin .
Haemoglobin	11.7	11.8	12
P.V.C	40.3	39	42.1
R.B.C Erythrocyte	6.8	6.5	7.0
Leukocyte WBC	11.8	10.2	10.9

Grain and fruit samples from the local markets in Najaf were collected to detect and identify fungi isolates associated with inappropriate storage and aflatoxins production. The results showed that all the samples (grain and fruits) obtained from the markets were contaminated with fungi to varying degrees, resulting in the production of aflatoxins.

The isolation and distribution of fungi in grain and fruits in Najaf is an innovative detection that reveals a display of fungi that are pathogenic to humans and animals. Ten fungal genera were detected from grain with incidence: *Aspergillus flavus* 15 (18.7%), *Aspergillus niger 12 (15%), Penicillium spp 7 (8.7%), Aspergillus terreus 7 (8.7%), Aspergillus fumigatus 7(8.7%), Alternaria spp. 10 (12.5%),, Aspergillus parasiticus 6 (7.5%) Fusarium 6 (7.5%), Penicillium chrysogenum 5(6.3), Mucor spp.2 (2.5%),and Rhizopus stolinfier 3 (5.5%)*.The occurrences of these suggest that these fungal isolates could be accountable for the grain contamination. *Aspergillus flavus* and *Aspergillus niger* were the two most frequently isolated genera. *A. flavus* and *A. niger* are known to produce aflatoxins and ochratoxin (Inam-ul-Haq et al.,2023). However, during the current study, the isolate *Aspergillus fumigatus* has proved to be the most prolific aflatoxin producer, and this may threaten the health of customers. Researchers have investigated three types of grain samples collected from the two important grain markets in Kano State. They isolated seven fungal species according to their morphological characteristics. The isolated species were identified as *Alternaria, Aspergillus flavus, Aspergillus niger, Cladosporium, Fusarium,* and *Rhizopus* species (Inam-ul-Haq et al.,2023).

The identification and spreading of fungi in spoiled fruits in local markets in Najaf is a finding that reveals a visible collection of fungi that are harmful to humans and animals. The recognized

fungal organisms associated with spoilt fruits in the study area include *Penicillium* spp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, and *Aspergillus parasiticus*, suggesting that these fungal organisms could be accountable for the fruit decay. This outcome is comparable with the study of (Okoji. and Isah., 2014).

According to earlier research, physical damage to fruits during processes like harvesting, storing, packing, and transporting them may increase post-harvest loss and raise the possibility of fungal contamination (Oguz. et al., 2000). Market surroundings that may boost contamination can be degraded by the low hygiene management system of the retailer, and microbially unsafe packaging, poor handling techniques, and unfavorable environmental conditions, such as a sanitary but unsafe marketing environment, are all examples of poor practices. Increased fruit loss due to microbial spoilage and the presence of some human pathogens could be a result of the issues Chronic aflatoxicosis can also be diagnosed using serum biochemical and hematological changes. Due to the negative impact that aflatoxin has on particular biochemical and hematological values, even very low concentrations of the substance are toxic to animal and human health (Basmacioglu et al., *2005)*.

In the present study, an isolate of *Aspergillus fumigatus* was selected for its highest productivity of afaltoxin. This is the first report of its kind performed using okra extracts as a mitigation agent to investigate the reduction of the negative impact of aflatoxin in mice. Hemoglobin, PVC, erythrocyte count, and leukocyte count levels were decreased in the group fed aflatoxin alone compared with the other groups. However, this decrease in hemoglobin, leukocytes, and erythrocytes was significant in the group fed aflatoxin alone when compared to the control, and a similar decline was also observed in the group fed okra extract and aflatoxin when compared to control values. In contrast, these parameters tended to rise in the same group when compared to values in the flatoxin-fed group. Aflatoxin was also found to reduce hematocrit, hemoglobin, erythrocyte, thrombocyte, and lymphocyte counts in a different study, and glucomannan (1 g/kg) was reported to mitigate these adverse effects of aflatoxin in broiler chickens (Abdel-Wahhab et al., 2002). Our results corroborated the claims recorded by (Yousef, *et al.* (2003), which displayed that the utilization of aflatoxin solely caused a decrease in hemoglobin concentration and total red blood cell counts that prompted leukopenia and normocytic anemia. There are numerous potential causes for this decline in hematological parameters, including hemopoietic cellular defects in aflatoxin, reduced iron binding capacity, and inhibition of protein synthesis as indicated by lower serum albumin (Kilany *et al., 2020)*.

CONCLUSION

Sixteen fungal species were isolated from grain and fruit samples obtained from the local markets in Najaf, Iraq. The isolated species were identified morphologically. These pathogenic fungi species associated with grain and fruit spoilage are of economic and public health significance. Erythrocyte, leukocyte count, PVC, and hemoglobin levels were decreased in the aflatoxin group compared with the other groups, and there was a reduction in similar parameters in the aflatoxin and okra groups compared to control values. On the other hand, these parameters tended to increase in the aflatoxin and okra extract groups compared to aflatoxin group values. In conclusion, the aflatoxin and okra group levels being between the control and aflatoxin groups have shown that okra extract at this level has a moderate effect on this aflatoxin dosage in respect of these parameters. Thus, it needs other studies on different aflatoxin and okra levels. As a result, the findings presented in this study contribute to the overall literature, and chronic intoxications can be diagnosed before clinical signs occur. The effectiveness of okra extract in achieving this outcome is believed to be important.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Hussein Ali Hussein contributed substantially to the conception and design of the study

Khawlah Abdallah Salman contributed to performing the experiment.

Athraa Harjan Mohsen contributed to analyzing the data.

Israa Harjan Mohsen provided the final approval of the version to publish.

All authors discussed the results and contributed to the final manuscript.

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