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Determination of Ochratoxin A in sorghum in Khartoum, Gadaref and Kordofan states in Sudan.

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ABSTRACT

Ochratoxin A (OTA) has been shown to be a potent nephrotoxic, hepatotoxic, and teratogenic compound. In farm animals, the intake of feed contaminated with OTA affects animal health and productivity, and may result in the presence of OTA in the animal products. Strategies for the control of OTA in food products require early identification and elimination of contaminated commodities from the food chain. The objective of this study is to investigate the presence of ochratoxin A (OTA) in sorghum samples collected from three states of Sudan (Khartoum, Gadaref and Kordofan) and to quantify the amounts of ochratoxin A in sorghum, a simultaneous analytical method was developed, using system High Performance Liquid Chromatography (HPLC) with fluorescence detection. The results showed that 5 out of 30 (16.7%) of the sorghum samples were contaminated by ochratoxin A at concentration ranging between 0.4 and 0.6 $\mu\text{g kg}^{-1}$ with recovery 98% at spiking level 2 $\mu\text{g kg}^{-1}$ and with detection limit (LOD) of 0.4 $\mu\text{g kg}^{-1}$.

Keywords: *Ochratoxin A, sorghum, HPLC, Khartoum, Gadaref, Kordofan.*

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INTRODUCTION

Ochratoxin A (OTA) is one of the several fungal mycotoxins that have aroused significant public concern worldwide. The disease caused by OTA exposure is known as *ochratoxicosis*, and the primary target is the kidney. Epidemiological studies show that OTA may be involved in the pathogenesis of different forms of human nephropathies, including kidney cancer (1–3). Tumor incidence data from long-term animal studies also provides reasons for concern about the effect of OTA exposure on the human population. Thus, OTA was classified as a possible carcinogen (Group 2B) to humans by The International Agency for Research on Cancer (IARC) (4). The mechanism of action of OTA is unclear. Recent reports suggest that oxidative pathway and genotoxicity are the key points for both nephrotoxicity and carcinogenicity (3). OTA may be encountered in a host of common foodstuff and beverages. The highest reported occurrence of OTA was found in cereal grains, and to a lower extent in other foodstuff of plant origin (*i.e.*, wine, coffee, beer, spices and chocolate). Moreover, considering that mycotoxins can be transferred through the food chain, OTA can also be found in tissues and products of animal origin, pork and poultry, and dairy products, among others (5,6). OTA is a secondary toxic metabolite produced mainly by some strains of *Aspergillus ochraceus* and *Penicillium verrucosum* species. These species can grow in different climates. *Aspergillus* are found in tropical regions, whereas *Penicillia* are common in temperate regions; and can grow when the temperature is as low as 5 °C (7). In general, OTA formation occurs mainly after harvesting on insufficiently dried cereal and cereal products. Factors influencing OTA production include environmental conditions, such as temperature and water activity, but also the type and integrity of the seeds. While *A. ochraceus* grows better in oilseeds (peanuts and soybeans) than in grain crops, such as wheat and corn, *P. verrucosum* may grow better in wheat and corn (8).

OTA occurrence in human food commodities of vegetal and animal origin has been recognized as a potentially global human health hazard. Several detailed risk assessments have linked kidney damage incidence to estimated OTA consumption in the diet (9). OTA is associated with the Balkan Endemic Nephropathy and was also linked to human renal disease (10,11). A general maximum OTA limit of 5 µg/kg in cereals and 3 µg/kg in cereal products was proposed by the World Health Organization (7). In Sudan sorghum is the staple cereal for the majority of the population, it is cultivated in vast agricultural areas in the Sudan producing thousands of tons. Most of the production is used locally as staple diet.

MATERIALS AND METHODS

SAMPLES SOURCE

All samples analyzed have been collected randomly from market of three states in Sudan (Khartoum, Gadaref and Blue Kordofan).the samples size was 0.5-1 kg. The samples were kept at freezer till tested. At the time of analysis samples were brought up to room temperature (12).Extraction, clean up and determination of ochratoxin A was done using AOAC official method 991.44 (13).

EXTRACTION AND CLEANUP

In brief; sorghum powder (50 g) was transferred into 500 mL conical flask and 250 mL chloroform, 25 mL 0.1 M phosphoric acid were then added. The flask was securely stopper and shaken on a wrist action shaker for 30 min and filtered through filter paper cover with diatomaceous earth. About 50 mL of the filtrate was mix with 40 ml hexane and loaded into chromatography column contain mix 2g diatomaceous earth with 1ml 1.25% sodium bicarbonate then removed ochratoxin A with 75ml acetic acid: chloroform (1:99) and collect it and evaporated to dryness in steam bath .The dry extract was dissolved with 500 µL mobile phase and injected into High Performance Liquid Chromatography (HPLC).

Separation and detection

The chromatographic conditions were as follows:

- Column type and size: C18; 250 X 4.6 mm I.D.; 5 micron particle size
- Temperature: ambient temperature 25°C
- Fluorescence detector: 333 and 460 nm as wavelengths for excitation and emission, respectively
- Mobile phase: acetonitrile :water :acetic acid (99: 99: 2)
- Flow rate: 1 mL min⁻¹
- Injection Volume: 20 µL

Calibration curve was determined, using series of dilutions containing 0.25, 0.5, 2.5 and 5 ng/ml of ochratoxin A. The correlation factor R² was 0.9936.

RESULTS AND DISCUSSION

The results of the present investigation shown in table 1. Ochratoxin A was detected in 5 out of 30 (16.7%) sorghum samples with an average concentration of 0.08 µg kg⁻¹. The samples that have been contaminated by Ochratoxin A are from Gadaref (three samples) and Kordofan (two samples) with average of 0.08 and 0.48 from total samples assayed and positive samples respectively .the average of the contaminated samples from Gadaref are 0.12 and 0.4 from total samples assayed and positive samples respectively, and the average of the contaminated samples from Kordofan are 0.12 and 0.6 from total samples assayed and positive samples respectively.

These contamination levels by ochratoxin A are alarmingly low in view of the fact that the Tolerable Daily Intake (TDI) set by the European Union (EU), the Scientific Committee for Food (SCF) is 5 ng kg⁻¹ bw/day A(14).

Table 1. Prevalence of Ochratoxin A in the sorghum samples

state	No. of samples			Average of Ochratoxin A from assayed	Average of Ochratoxin A from positive
	Assayed	Positive	%Positive		
Khartoum	10	0	0	0	0
Gadaref	10	3	30	0.12	0.4
Kordofan	10	2	20	0.12	0.6
Total	30	5	16.7	0.08	0.48

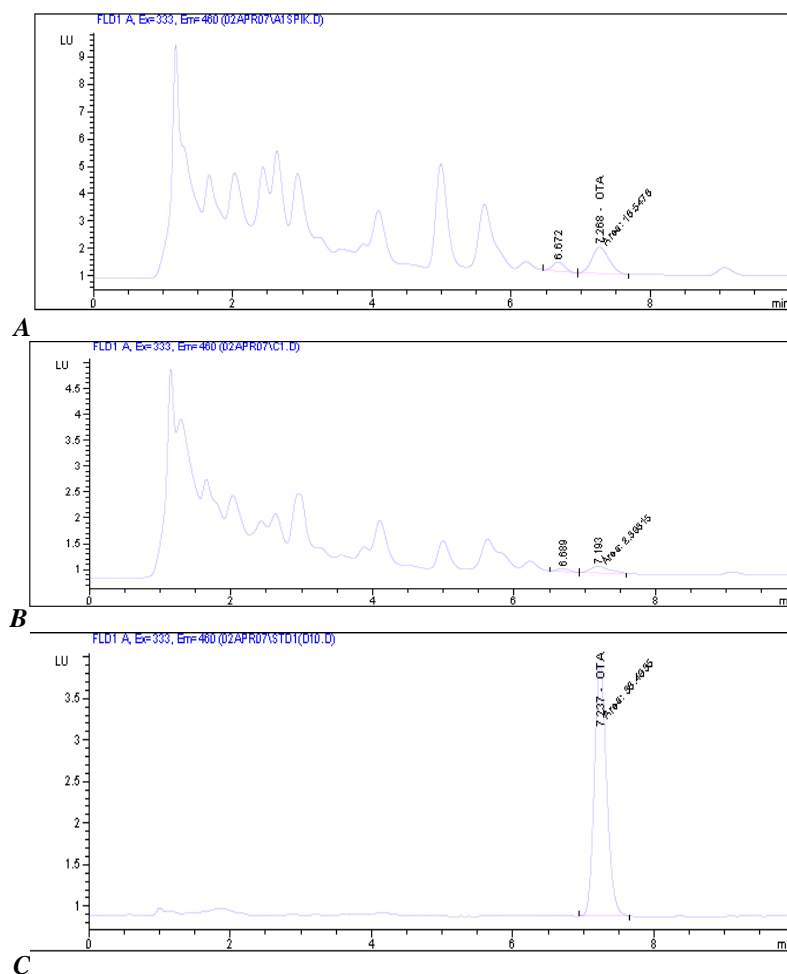


Figure 1. A (Spick sample). B (contaminated sample), C (ochratoxin A standard)

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