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Presence of ochratoxin A in human urine from Al-Jafara region, Libya: A preliminary study

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Abstract: A total of 85 samples of human urine were analyzed in this preliminary study for the presence of ochratoxin A using immunoaffinity column clean up (Ochra Test column) and HPLC, among which 65 samples were collected from the poly-clinical hospital in Alzahra city- Al-Jafara region for people attending nephropathy clinical for unknown causes, 5 samples from dialysis patient and 15 samples from healthy people in Jan-Feb 2009. From 85 participants involved in this study; 41 were females (48.23%) and 44 were males (51.76%). Females aged between 24 and 69 years old and males aged between 26 and 77 years old. Analysis showed presence of ochratoxin A in urine of nephropathy patients in 4 samples from females (9.75%) with concentrations ranging from 0.49 to 0.68 µg/l and an average concentration of 0.57 µg/l in the positive samples. 9 samples from males were positive (20.45%) with concentrations ranging from 0.20 to 2.60 µg/l and an average of 0.70 µg/l. The lowest concentration recorded in this study was 0.20 µg/l and the highest was 2.60 µg/l in male samples. Ochratoxin A was not detected in urine samples of dialysis patient and healthy samples at detection limit of 0.20 µg/l. In humans, ochratoxin A is metabolized very slowly with a half life of more than 35 days and presence of this toxin in human urine is considerd a serious health problem which results from consumption of food contaminated with ochratoxin A.

Key words: ochratoxin A, urine, nephropathy, Libya

Introduction

Ochratoxin A (OTA) is the first group of mycotoxins discovered after Aflatoxins as low molecular weight, toxic chemical product formed as secondary metabolite by a few fungal species that readily colonize crops and contaminate them (Sherif *et al*. 2009, Turner *et al*. 2009). It is produced by *Penicillium verrucosum*, *Aspergillus ochraceus*, *A. niger* and *A. carbonarius* (Juan *et al*., 2008; Bento *et al*. 2009). Ochratoxin A is a frequent contaminant of various food products such as cereals, cereal derived products, coffee, dried fruits, spices and beans (Zaied *et al*. 2009) as well as of animal products due to transfer from contaminated feeds (Kabak 2009), and after ingestion. Ochratoxin is frequently found in human blood due to the long time of its elimination (35days in serum) as consequence of its binding almost completely(>99%) to serum proteins, as well as its reabsorption from urine (Ringo *et al.* 2006, Munoz *et al*. 2009, Erkekoglu *et al*. 2010). This makes ochratoxin A to be the most usually detected mycotoxin in human blood and urine (Duartes *et al*. 2010, Pena *et al*. 2006). In humans, consumption of ochratoxin A contaminated food results in nephrotoxic, teratogeni, genotoxic, carcinogenic and immunosuppressive effects (Zinedine *et al*. 2010).

Molecular toxicity of ochratoxin A results from competition with phenylalanine for protein synthesis, promotion of lipid peroxidation, inhibition of mitochondrial ATP production, as well as production of DNA adducts (Kamp *et al*. 2005, Dinis *et al*. 2007). Ochratoxin A suspected to cause the Balkan endemic nephropathy (BEN), a familial chronic tubulo-interstitial disease with a slow progression to terminal renal failure, affects people living in rural areas in south eastern Europe (e.g. Romania, Bulgaria, Bosnia, Serbia & Croatia). One of its most peculiar characteristics is a strong association with upper urothelial cancer (Stefanovic *et al*. 2009) and the Tunisian chronic interstitial nephropathy (CIN) of unknown causes (Zaied *et al*. 2009). As far as human concern the International Agency for Research on Cancer (IARC) classified ochratoxin A as a possible carcinogenic agent for humans (group 2B) (IARC 2002).

Numerous reports from many parts of the world described high frequency of human blood and urine ochratoxin A contamination at various concentrations. This indicates the widespread of human exposure to this mycotoxin. However, in research study by Pena *et al*. (2006) for the follow up of ochratoxin A in 60 urine samples of inhabitants from Coimba city- Portugal in order to evaluate population contamination and the presence of ochratoxin A showed that 42 samples

(70%) were contaminated at concentration range between 0.021 and 0.105 µg/l.

The aim of this study was to estimate contamination of human urine from Al-Jafara region-Libya with ochratoxin A and to add new data which may be useful for several studies conducted in different parts of North Africa.

Material and Methods

Urine Samples

Eighty five (85) samples of human urine were used in this study, of which 65 samples were collected from the poly-clinical hospital in Alzahra city- Aljafara from people attending nephropathy clinical for unknown causes, 5 samples from dialysis patients and 15 samples from healthy people during Jan-Feb, 2009.
From 85 participants involved in this study, 41 were females (48.23%) and 44 were males (51.76%). Females aged between 24 and 69 years old and males aged between 26 and 77 years old. From each more than 15 ml urine samples were obtained and stored at 4 ^{o}C until analysis.

Urine sample preparation and immunoaffinity clean-up

For analysis of ochratoxin A in human urine, ten ml of human urine diluted with 10 ml of 5% sodium bicarbonate in distilled water were mixed and filtered through Whatman No 1filter paper. 10 ml of filtered sample were cleaned up through the Ochra Test immunoaffinity column (Ochratest, Vicam) at a flow rate of about 1 drop per second. After passage of the sample, the column was washed twice with 10 ml distilled water at a flow rate 1-2 drops per second. After that 3 ml of HPLC grade methanol were passed at a rate of one drop/second, then the eluate was evaporated to dryness under nitrogen gas at 50 ^{o}C and reconstituted with 200 µl of the HPLC mobile phase (Pena *et al*. 2006, Manique *et al*. 2008).

HPLC determination of ochratoxin A

Ochratoxin A in urine samples was quantified by reverse phase HPLC with fluorescence detection (Shimadza-LC 10 A series) according to the method of (Pena *et al*. 2006). 50 µl of the reconstituted extract were injected into the chromatographic apparatus by full loop injection system. The mobile phase consisted of a mixture of acetonitrile/water/acetic acid (99:99:2) at a flow rate of 0.8 ml/min and temperature of 40 ^{o}C. Quantification of ochratoxin A was performed with a fluorescence detector with excitation wavelength 333 nm and emission wavelength 460 nm. A four-point calibration curve (0-2-5-10 µg/l) was established. The calibration curve was linear (0.9996) with precision less than 5%. The Quantification of ochratoxin A concentration was performed by measurement of peak areas at ochratoxin A retention time at 8 min and comparison with the calibration curve.

Recovery experiments were performed in triplicate by spiking urine samples, known to be ochratoxin A-free, with ochratoxin A at levels 2-5 and 10 µg/l. Average recoveries ranged from 87 to 93% with the detection limit 0.02 µg/l. Identification was confirmed by methyl ester derivatization according to the method described by Pena *et al*. (2006).

Results and Discussion

In this study, a total of 85 samples of human urine were analyzed for the presence of ochratoxin A using immunoaffinity column clean up (Ochra test column) and HPLC.

The results showed the presence of ochratoxin A in 13 samples (15.29%) from the total samples used and 20% of the nephropathy samples at a concentration range from 0.20 to 2.60 µg/l. Analysis of the female urine samples indicated that ochratoxin A was present in 4 samples of nephropathy patients (9.75%) only with concentration range from 0.49 to 0.68 µg/l and average concentration of 0.57 µg/l in positive samples. In case of males 9 samples (20.45%) with concentration range from 0.20 to 2.60 µg/l and average concentration of 0.70 µg/l in positive samples were obtained with the lowest concentration recorded in this study at 0.20 µg/l and the highest concentration was 2.60 µg/l. Ochratoxin A was not detected in urine samples of dialysis patients and healthy people at the detection limit 0.020 µg/l (Table 1).

This preliminary study is the first study in Libya which revealed the presence of ochratoxin A in human urine. The widespread contamination of foodstuffs by mycotoxins, such as ochratoxin A (OTA) has made the monitoring of human contamination levels essential (Duarte *et al*. 2009).

The results showed the presence of ochratoxin A in 13 urine samples of people attending nephropathy clinical hospital for unknown cause. Results indicated that humans are continuously exposed to ochratoxin A from consumption of contaminated food and this causes nephropathy. The other 52 urine samples of nephropathy showed absence of ochratoxin A in the urine. Also, the urine samples of dialysis showed absence of ochratoxin A. Several research work indicated the presence of ochratoxin A in wheat, barley, rice, spices and coffee used for human consumption (Noonim *et al*. 2008, Ghali *et al*. 2009,

Table 1: Ochratoxin A concentration (µg/l) in positive samples of urine.

Sex	Age (year)	Concentration (µg/l)
Female	40	0.49
Female	69	0.68
Female	60	0.55
Female	24	0.56
Male	76	2.60
Male	33	0.65
Male	26	0.40
Male	42	0.20
Male	70	1.00
Male	50	0.80
Male	77	0.21
Male	50	0.25
Male	26	0.20

Zaied *et al*. 2009, kabak *et al*. 2009), which are considerd to constitute the base of diet in Libya.

Several studies are in agreement with this preliminary study for the presence of ochratoxin A in urine. The ochratoxin A (OTA) content of urine samples from 88 healthy humans living at five settlements in three counties of Hungary was determined by Fazekas *et al*. (2005) and the contamination detected in 61% of the samples in an average concentration of 0.013 µg/l with the range of 0.006-0.065 µg/l. In another study for monitoring of ochratoxin A exposure in Portuguese population in five cities through nationwide urine survey in winter 2007 by Durate *et al*. (2010), a total of 155 morning urine samples were collected and the results showed the contamination incidence of 92.2% with the mean concentration of 0.018 µg/l. In research work for quantification of ochratoxin A in 60 morning and afternoon portions of urine from30 healthy individuals from Coimbra city in Portugal and 62 samples from 31 healthy individuals from Valencia city, Spain. The results also showed presence of ochratoxin A in 13 and 14 samples of morning and afternoon portions respectively, with concentrations range from 0.008 to 0.208 µg/l in city of Coimbra and presence of ochratoxin A in 25 and 26 samples of morning and afternoon portions, respectively with concentrations range from 0.007 to 0.124 µg/l in city of Valencia (Manique *et al*. 2008). In addition, ochratoxin A occurrence and level in 233 human urine samples, collected from 4 different regions of Turkey were analyzed by Akdemir *et al*. (2010) and showed that 83% of the samples were contaminated with the highest concentration of 75.60 µg/l and average concentration 14.34 µg/l. Results of 13 urine samples from healthy volunteers in Dortmund, Germany examined by Munoz *et al*. (2009) showed the presence of ochratoxin A with the mean of 0.07 µg/l.

It is well established in humans that ochratoxin A is metabolized very slowly with a long elimination half life (35days in serum) as consequence of its binding almost completely(>99%) to serum proteins ((Ringot *et al*. 2006, Munoz *et al*. 2009, Erkekoglu *et al*. 2010). Presence of this toxin in human urine in this preliminary study indicates a serious health problem in area of study since this toxin is recognized as a possible carcinogenic agent to humans and animals by International Agency for Research on Cancer (IARC, 2002) which results from consumption of food contaminated with ochratoxin A.

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