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Presence of ochratoxin A in some food in Al-Jafara region-Libya Preliminary study

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Abstract: Eleven food samples and four coffee samples of several kinds and trade marks were collected from different super-markets in Al-Jafara region, Libya and analysed for the presence of ochratoxin A, using immunoaffinity column clean up (Ochra test column) and HPLC. The samples were of different cereal products and coffee consumed regularly and normally. The results indicated that eight food samples (72.72%) were contaminated by ochratoxin A with the lowest concentration (0.59µg/kg) in couscous (national production) and the highest concentration (15.50µg/kg) in couscous (imported). Four food samples (36.36%) contained ochratoxin A above the Libyan and European specifications which allow 3µg/kg. Of the coffee samples, ochratoxin A was found in 2 samples of Arabic coffee (50%) and the highest concentration was present in Arabic coffee (locally processed) with concentration reaching 70.16 µg/kg which is highly above the Libyan and Europe Union regulations which allow 5 µg/kg for Arabic coffee. The other food samples which showed presence of ochratoxin A had concentrations of 10.70, 1.49 and 1.13 µg/kg for macaroni (national production), 3.25 µg/kg for rice (imported), 4.80 µg/kg for wheat flour (National production), 1.89 µg/kg for wheat flour (imported), 3.32 µg/kg for Arabic coffee and 15.50 µg/kg for couscous (imported). The other three samples of food (27.27%) which consisted of one imported macaroni sample, two samples of imported rice and two samples of instant coffee (50%) showed absence of ochratoxin A at the detection limit below 0.02 µg/kg. Presence of ochratoxin A in foods considered a serious problem for human health where this toxin is recognized as possible carcinogenic to both human and animal health by International Agency for Research on Cancer.

Key words: ochratoxin A, food, macaroni, wheat flour, Arabic coffee, couscous, Libya

Introduction

The filamentous fungi grow rapidly on a variety of natural substrates at some stage of growing, harvesting, production, transport or storage (Noonim *et al.*, 2008), under appropriate temperature and moisture condition and produce mycotoxins. These toxins are secondary metabolites with relatively small molecular weight (Turner *et al.*, 2009). They are produced by many species of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria* spp. and known to be associated with human and animal disease (Zinedine and Manes, 2009). Recent studies estimate that mycotoxins contaminate 25% of the food crops and account for more than $ 1.4 billion in economic losses in the United State alone (Bingham *et al*., 2004). Ochratoxin A (OTA) is one of the most important mycotoxins of worldwide concern for human health due to its implication in a diverse range of toxicological effect (Kumar *et al*., 2008; Astoreca *et al*., 2009). Ochratoxins are secondary metabolites of fungi, containing dihydroisocoumarin moiety linked to a L phenylalanine (Ringot *et al*., 2006). These toxins are produced by *P. verrucosum*, *A. ochraceus*, *A. carbonarius* and *A. niger* (Juan *et al*., 2007; Batista *et al.*, 2009; Duarte *et al*., 2010) in cereals, cereal derivatives, coffee, dried fruits, spices and beans as well as in animal products by transfer through contaminated feeds (Kabak, 2009; Zaied *et al*., 2009). The International Agency for Research on Cancer classified ochratoxin A as possible carcinogenic for both humans and animals (group 2B) (IARC, 2002), and also ochratoxin A was suspected to be involved in the Balkan Endemic Nephropathy (BEN), a fatal chronic kidney disease of people observed in rural areas in south eastern Europe (Romania, Bulgaria and Bosnia) and characterized by progressive renal fibrosis in human (Erkekoglu *et al*., 2010). Ochratoxin A has nephrotoxicity, teratogenic toxicity, immunotoxicity, genotoxicity, and carcinogenic properties and possibly neurotoxic properties (Zinedine *et al*., 2010). Several studies showed presence of ochratoxin A in food used for human consumption. In analytical study for detection of ochratoxin A levels in 63 samples of infant formulae, follow on formulae and baby foods marketed in Ankara, Turkey, ochratoxin A levels in the baby food samples positive for ochratoxin A were in ranges of 0.06–6.04 µg/kg (Baydar *et al.*, 2007). In analytical study for detection of ochratoxin A levels in 68 samples of breakfast and infants cereals products collected from different supermarkets and pharmacies in Rabat, Morocco by Zinedine *et al*., (2010) showed that four samples of breakfast cereals (5.8% of total samples) were found contaminated with 5.1 and 224.6 µg/kg OTA. In research work on the presence of several mycotoxins including ochratoxin A in 209 samples of different groups of food widely consumed by the Tunisian population including spices, dried fruit, sorghum and rice

showed presence of ochratoxin A in 59.8% of the samples with a mean level of 3.5 µg/kg (Ghali *et al*., 2008). Other investigation in Tunisia, carried out by Zaid *et al*., (2009) for the presence of ochratoxin A in widely consumed cereals include 110 wheat samples, 103 barley, 113 sorghum and 96 rice samples during the year 2004-2005 revealed contamination with incidence of 38%, 40%, 38% and 28% with average concentrations of ochratoxin A 55, 96, 44 and117 µg/kg respectively.

The aim of this preliminary study is to screen some foods consumed regularly and normally by most people in Al-Jafara area-Libya for the presence of ochratoxin A.

Materials and Methods

Food samples

Eleven (11) food samples and four (4) coffee samples of several kinds and trade marks were collected from different markets in the area of Al-Zahra city-Al-Jafara – Libya. They consist of 3 samples of macaroni of 500 gram each (national production), one sample of macaroni of 500 gram (imported), 3 samples of rice of 3 kg each (imported), one sample of wheat flour of 3 kg (national production), one sample of wheat flour of 3 kg (imported), 2 samples of Arabic coffee of 500 gram each and one sample of couscous of 3 kg (locally processed), 2 samples of instant coffee of 500 gram each and one sample of couscous of 3 kg (imported).

All food samples were collected randomly a few days before analysis with valid expiry date for consumption and available normally for human consumption in different markets.

Food sample preparation and immunoaffinity clean-up

For determination of ochratoxin A in food, 50g of mixed samples were homogenized in a blender with 100 ml methanol: water (80:20) for one min and filtered through Whatman No. 1 filter paper. After filtration, a 10 ml aliquot was completed to a volume of 50 ml with distilled water. This solution was then filtered a second time using filter paper Whatman No 1 and 10 ml of this filtrate was applied to an immunoaffinity column (Ochratest, Vicam) at a flow rate of one drop/second. After passage of the sample, the column was washed twice with 10 ml distilled water and 3 ml of HPLC grade methanol was used to elute ochratoxin bound to monoclonal antibodies in amber glassware, at a flow rate of one drop/second. The elute was evaporated to dryness under nitrogen gas at 40 °C and reconstituted with 200 µl of the HPLC mobile phase for quantification. The mobile phase consisted of a mixture of acetonitrile/water/acetic acid (99:99:2) (Alarcon *et al*., 2006; Sugita-Konishi *et al*., 2006).

HPLC determination of ochratoxin A

Ochratoxin A in food samples quantified by reverse phase HPLC with fluorescence detection (Shimadza-LC 10 A series) according to the method of Alarcon *et al*. (2006). Fifty µl of 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 °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 blank food samples with ochratoxin A at levels 2-5 and 10µg/kg. Average recoveries ranged from 87 to 93% with the detection limit of 0.02µg/kg. The identification was confirmed by methyl ester derivatization according to the method described by Pena *et al*. (2006).

Results and Discussion

The present results indicated the presence of ochratoxin A in eight samples (72.72%) with the lowest concentration of 0.59 µg/kg in couscous (national) and the highest of 15.50 µg/kg in couscous (imported). Four samples (36.36%) of the positive ones were above the Libyan specification (Libyan specification No 683/2009) and EU regulation (Commission Regulation, EC No. 1881/2006) which permit 3 µg/kg for food used for human consumption. The other three samples of food (27.27%) which consisted of one sample macaroni (imported), two samples of rice (imported) show absence of ochratoxin A at the detection limit below 0.02 µg/kg (Table 1). The presence of ochratoxin in the samples used in this preliminary study could be due to inadequate storage conditions or low quality of food. Our results are in agreement with several studies in the world and North Africa for the presence of ochratoxin A in food used for human consumption. In a study of human exposure to ochratoxin A in selected population in Egypt, a total of 140 samples of cereals (rice and macaroni), legumes, dried fruits, dairy products and meat were collected randomly from Egyptian countryside and the results indicated presence of ochratoxin A in 33.56% of total samples and the highest ochratoxin A levels were found in cereals with the concentration range from 18 to 421 µg/kg (Zohir and Salim, 2006). Evaluation of ochratoxin A exposure degree in two Portuguese cities evaluated by Duarte *et al*. (2010)

using 168 samples of wheat and maize bread consumption during winter 2007 showed that 84% of samples were contaminated with maximum level of 3.85 µg/kg. One hundred (100) samples of rice purchased from retail markets in five different cities in Morocco from January to October 2006 were surveyed by Juan *et al*. (2008a) for the presence of ochratoxin A and the analytical result showed a frequency of contamination of 26% of the total rice

Table 1: Ochratoxin A concentration (µg/kg) in the food samples

Food sample	**Origin**	**Ochratoxin A (µg/kg)**
Macaroni	National production	10.70*
Macaroni	National production	1.49
Macaroni	National production	1.13
Macaroni	Imported	<0.2
Rice	Imported	3.25*
Rice	Imported	<0.2
Rice	Imported	<0.2
Wheat flour	National production	4.80*
Wheat flour	Imported	1.89
Arabic coffee	Locally processed	3.32*
Arabic coffee	Locally processed	70.16*
Instant coffee	Imported	<0.2
Instant coffee	Imported	<0.2
Couscous	National production (home-made)	0.59
Couscous	Imported	15.50*

*Above the Libyan and European regulations; Detection limit= 0.02 µg/kg.

Samples analysed and the concentration ranged between 0.08 and 47 µg/kg. Investigation carried out by Juan *et al*., (2008b) for presence of ochratoxin A in a total of 61 samples of bread from central zone of Portugal showed 12.9% and 70% of wheat and maize bread respectively contaminated with ochratoxin A with concentration range between 0.02 to 1.19 µg/kg. Samples (180) of high consumption food commodities from various regions of Tunisia were analysed by Ghali *et al*. (2009) to determine ochratoxin A contamination levels, and performed analysis indicated that 45% of monitored samples were contaminated with levels ranging from 0.11 to 33.9 µg/kg, and the most contaminated commodities were barley, sorghum and wheat. In 30 samples of wheat flour, 30 samples of corn starch and 31 samples of rice on Chilean market, carried by Vega *et al*. (2009) revealed about 70%, 63% and 50% of flour, corn starches and rice respectively were positive for ochratoxin A. In Greece, study conducted by Villa and Markaki (2009) in 55 samples of breakfast cereals from Athens markets showed that 60% of samples contained ochratoxin A with the mean concentration 0.18 µg/kg. The natural occurrence of ochratoxin A for 60 samples of cereals in Morocco (20 of each of corn, barley and wheat) indicated that the average levels of ochratoxin A were 1.08, 0.42 and 0.17 µg/kg for corn, wheat and barley respectively (Zinedine *et al*., 2006). A total of 100 samples of commercial bread purchased from January to October 2006 from retail baking shops in five cities in Morocco were surveyed for presence of ochratoxin A and the analytical results showed that 48% were positive for ochratoxin A with levels ranged between 0.14 and 149 µg/kg with average levels of 13 µg/kg and 26% of positive samples exceeded the maximum of 3 µg/kg set by EU (Zinedine *et al.,* 2007). In study conducted by Abdulkader *et al.* (2004) on mycotoxins in food available in Qatar including ochratoxin A in 106 samples of various kinds of food products collected from the markets, 11 samples were found contaminated with ochratoxin A in the range of 0.20 – 4.91 µg/kg. In wheat bread samples collected in winter 2007 from the city of Algarve and Barganca region-Portugal by Bento *et al*. (2009), 60% and 50% of the samples contained ochratoxin A with maximum concentration of 0.49 and 0.43 µg/kg respectively.

In the coffee samples used in this study, ochratoxin A was found in 2 samples of Arabic coffee (50%) and one of them showed a concentration of 70.16 µg/kg which is highly above the Libyan specification (Libyan specification No 683/2009) and European Union regulation which allow 5 µg /kg for Arabic coffee (Commission Regulation, EC No 1881/2006). The presence of this toxin could be due to the inadequate roasting process or bad storage conditions of the coffee or heavy contamination of coffee seed before roasting. Coffee roasting can remove a very significant percentage of ochratoxin A, depending on the roasting process, destruction can be from 8% to 98% (Ferraz *et al*., 2010) where the instant coffee was found free from ochratoxin A. In 64 samples of coffee beans collected in 2006-2007 from Thailand, by Noonim *et al.* (2008) showed that 98% of the samples were contaminated with ochratoxin A in levels of 0.6 – 5.5 µg/kg. Ochratoxin A was detected in 89 of coffee samples (31%) at concentrations of 0.1–5.0 µg/kg and 25% of the samples at concentrations above 5.0 µg/kg. Of the 40 bean samples collected from farms of southern Minas Gerais municipalities, Brazil, and analysed, 58% were infected with potentially ochratoxigenic fungi but only 22% of them were contaminated with ochratoxin A at levels that varied from 0.47 to 4.82µg/kg, with an average contamination level of 2.45 µg/kg (Batista *et al* 2009).

Consumption of food products contaminated with toxigenic fungi can possess serious health hazards to human and animals. Since the consumption of mycotoxins-contaminated diet may induce acute and chronic effects resulting in a teratogenic,

carcinogenic (mainly for liver and kidney), estrogenic or immunosuppressive effect on animals and man (Erkekoglu *et al*., 2010). Molecular toxicity would result from competition with phenylalanine for protein synthesis, promotion of lipid peroxidation, inhibition of mitochondrial ATP production as well as production of DNA adducts (Marin-Kuan *et al*., 2008).

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