

Ochratoxin A in Cow's Milk Collected from Cattle Farms of Golestan Province

Sakine Tale Hel Abad (MSc)

Department of Biochemistry, Islamic Azad University, Fars Science and Research Branch, Shiraz, Iran

Hamidreza Joshaghani (PhD)

Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Mojgan Nejabat (MSc)

Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Hadi Rahimzadeh (MSc)

Environmental Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Farhad Niknejad (PhD)

Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Mohammad Reza Kiaie(BSc)

Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding author: Farhad Niknejad

Email: fniknezhad@yahoo.com

Tel: +989112758801

Address: Department of Medical Mycology, Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

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ABSTRACT

Background and Objective: Ochratoxin is a fungal toxin produced by *Penicillium verrucosum* and some *Aspergillus* species. Ochratoxin is usually found in grains, cereal products and also animal feed of livestock. The aim of this study was to measure the level of Ochratoxin in pasteurized milk samples of Golestan Province, Iran.

Methods: Overall, 38 milk samples were collected from East and West of the Golestan province in accordance with standards 326 and 419 of the Institute of Standards and Industrial Research of Iran. The level of Ochratoxin was measured by ELISA method.

Results: The mean level of Ochratoxin A in 20 raw milk samples collected from the West of the Province was 3.32 ± 3.76 ng/ml. The mean level of Ochratoxin A in 18 raw milk samples collected from the East was 6.02 ± 4.42 ng/ml. Ochratoxin A levels in most samples were higher than the limits established by the European standards.

Conclusion: Based on the results of this study, Ochratoxin level of 84.2% and 52.6% of the samples from the West and East of the province are higher than the allowed limits (2 ng/ml), respectively.

Keywords: Ochratoxin, ELISA, milk, Golestan province.

Introduction

Mycotoxins are compounds with a low molecular weight (700 Daltons) that are produced in the final stages of growth of some fungi. These are often considered as secondary metabolites with a limited distribution and are not necessarily present in all species of a family. Ochratoxin is a fungal toxin produced by *Penicillium verrucosum* and some *Aspergillus* species. The risk of mycotoxin contamination is a major concern in the field of food safety of grains and other crops. Crops' vulnerability to this threat, is not the same. Mycotoxins contaminate peanuts, cereals (maize, rice, wheat, barley and oats), spices (black pepper, ginger, Indian nutmeg) and some other agricultural products. Human exposure to these toxins in areas where people consume damaged grains by either insects or molds, are at the highest level. Several studies have shown the nephrotoxic, immunotoxic, teratogenicity, carcinogenicity and possible neurotoxic and genotoxic properties of Ochratoxin A (OTA) (1). Ochratoxin in animals, especially pigs is associated with nephropathy, and if used in high amounts by humans will lead to Balkan endemic nephropathy - a chronic renal disease, mostly observed in the Balkans. It is also accompanied by increased incidence of tumors at the upper urinary tract (Samson 1994)(2). Ochratoxin have different toxic effects on various biological functions. Major toxic effects include inhibition of ATP and protein synthesis and lipid peroxidation (3). Acute and subacute toxicity of OTA via inhibition of phenylalanine-tRNA synthetase results in reduced protein synthesis (4). In addition to inhibition of protein synthesis, RNA and DNA synthesis are also affected and enzymes such as phosphoenolpyruvate, Carbonyl reductase C (5), succinate-cytochrome and succinate dehydrogenase (6) are inhibited. Inhibition of the enzymes associated with the synthesis of proteins, RNA and DNA may be a cause for cancer in people infected with OTA. Other toxic effects of OTA include cardiac and liver tissue abnormalities, defects in clotting factors accompanied with bleeding and thrombosis in spleen, brain, liver, kidney and heart (7). According to the results of various studies on the side effects and toxicity of ochratoxin, the

International Agency for Research on Cancer (IARC 1993) classified OTA as a human carcinogen (class 2B). Presence of ochratoxin in human milk increases the risk of infection in newborns. It is chemically stable and the temperature normally used for food preparation has no significant effect on its 35-day half-life in humans (Bullerman and Bianchini, 2007) (8), making it to have an average concentration of 0.5 nmole/l in blood (Skaug 2003) (9). Consumption of cow's milk is considered as one of the input sources of OTA. Given the limited biological changes in the gastrointestinal tract microflora to metabolize the toxin, a relatively high residual amount of ochratoxin can apply its toxic effects. The aim of this study was to measure the level of Ochratoxin in pasteurized milk samples of Golestan Province, Iran.

Material and Methods

In this study, 38 milk samples were obtained from collection stations of milk pasteurization plant of the Golestan province, Gorgan, Iran. Samples were collected from the whole province with 18 and 20 samples from the East and West of the province, respectively. Samples were collected according to the standards 326 and 419 of Iran's Institute of Standards and Industrial Research. Samples were kept in clean closed dishes in the fridge at -20 °C at laboratory of the Golestan University of Medical Sciences. For sample preparation, first the milk samples were passed through filter paper and 2 ml of milk were diluted with 2.5 ml HCL and 4 ml D-Hydro Chloromethane and then centrifuged for 5 minutes at 2000 g at room temperature. The upper layer was removed, 2 ml of the lower layer was taken and removed by mild nitrogen stream, D-Hydro Chloromethane and then completely dried. Next, 1 ml of the buffer provided within the kit, was added to the remaining and then 50 µl of phase 5 solution were added to the wells of the ELISA plate. The remaining steps were done according to the kit manufacturer's protocol (EuroProxima Kit, Cat. No. 991OCH01MS-96). Non-parametric tests were used for data analysis since the data distribution were found as not normal using the Kolmogorov-Smirnov and Shapiro-Wilk tests.

Results

All information regarding the samples collected from different regions of the province are summarized in the chart below. The mean amount of OTA in the 20 investigated samples collected from the West of the province was 3.32 ± 3.76 ng/L, with minimum value of zero and maximum of 21.4 ng/ml. The mean amount of OTA in the 18 investigated samples collected from the East of the province was 6.02 ± 4.42 ng/L, with minimum value of zero and maximum of 14.3 ng/ml. According to the European standards, the maximum allowed amount of Ochratoxin in food and products for children is 2 ng/L. In the Western region of the province, 4 samples (15.8%) were within the allowed range and 16

samples (84.2%) were higher than the allowed limit. In the Eastern region, 8 samples (47.4%) were found within the allowed range and 10 samples (52.6%) were reported to be higher than the allowed limit. Overall, of the 26 samples above the limited range, 16 were related to the West (61.5%) and 10 samples (38.5%) were related to the East. However, the difference between the contaminated samples was not statistically significant (P-value = 0.05). Mean level of Ochratoxin in this region in high- and low-risk areas of cancer in the Golestan province was found as 5.95 ± 4.11 and 3.90 ± 4.24 ng/L, respectively. However, this difference was not statistically significant (P-value = 0.841).

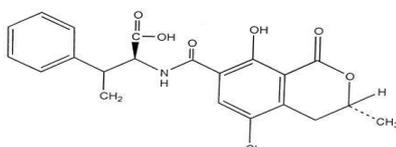


Figure 1- Chemical structure of ochratoxin A

Table 1- Information on samples collected

Location	Samples No	Positive samples*	Range (Mean)
East	18	10 (52.6%)	0-14.3 (6.02±4.42)
West	20	16 (84.2%)	0-21.4 (3.32±3.76)

Discussion

Based on the results of this study, the level of Ochratoxin presence in 84.2% of the samples taken from the West and 52.6% of collected samples from the East of the province, were higher than the allowed limit (2 µg/kg). El Zupir et al. studied 5 samples of cow's raw milk in Sudan and reported one sample (20%) with OTA level of 2.73 µg/L (10). Skaug in 1999, studied 47 samples of cow's milk and reported 6 OTA samples within the range of 11-58 ng/L (10). Kurtzman and Goto reported OTA level of 10-40 ng/L in cow's milk samples prepared in Sweden and Norway (11,12). Meanwhile, Valenta reported no OTA in 121 samples of cow's milk in Germany by two methods of ELISA and HPLC (13). Pattono studied 63 samples collected from industrial dairy farms and 20 samples from the traditional Italian livestock (cattle, goats, sheep), in 2011. Only 3 (4.8%) of the industrial dairy samples were found as OTA-positive, while OTA was not reported in any of the samples collected from the traditional livestock (14). Gonzalez-Osnaya et al. (2008) investigated 61 samples

of cow's milk from different regions of Valencia (Spain) using liquid chromatography and reported no OTA contamination in any of the studied samples (15). Boudra et al. in 2007, prepared milk samples from a number of cattle farms in Summer and Winter. Of the total of 256 samples that were tested, only 3 samples were detected with OTA at low range of 5-8 ng/L (16). Breitholtz reported 14% OTA contamination in the range of 10-40 ng/L among 36 samples of cow's milk in Sweden (17). The inconsistency of results of these studies with the present study regarding the level of mycotoxins contamination may be influenced by environmental factors (temperature, humidity and grain drying rate), appropriate hygiene practices and acceptable agricultural practices at all stages of the food chain. Moreover, good storage conditions can affect the susceptibility of products to fungal attacks in all stages of growth, storage and processing. Thus, the incidence of contamination in a particular product may be changed drastically from region to region and from year to year.

Conclusion

ELISA is considered as a relatively simple method that can be cost-effective to be used by food product factories or plants (such as grains, dried fruits, coffee and milk) to measure toxins. Given the importance of detecting these toxins, their removal from the production cycle before preparing the final product can prevent further costs of contamination for the manufacturer and eventually result in production of healthy products for consumers. Thus, the importance of identifying and screening of products that are vulnerable to fungal contamination should

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be taken into consideration by the healthcare systems, so that the necessary solutions to deal with these toxins are taken when required.

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Conflict of Interest

None to declare.