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Aflatoxin M_1 detection in milk in Mashhad-Iran by ELISA method

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ABSTRACT

The aim of this study was to evaluate aflatoxin M1 (AFM_1) contamination in milk samples in Mashhad in Iran. A total of 61 milk samples were collected from retail stores in June 2013. The occurrence and concentration range of AFM_1 in the samples were investigated by ELISA technique. AFM_1 was found in 53 (86%) of the examined milk samples by average concentration of 118.6ng/L and the contamination level ranging between 0-250ng/L. The concentration of AFM_1 in 30 (49%) samples was lower than the Iranian national standard (100ng/L) but the mycotoxin level in all the samples was lower than Food and Drug Administration limit (500 ng/L), and only in 9 (14.7%) of the samples, the concentration of AFM_1 was lower than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission. This situation must be considered as a food safety concern.

KEYWORDS

Aflatoxin M1;
Milk;
ELISA;
Mashhad.

INTRODUCTION

Mycotoxins are secondary metabolites of molds, which are associated with certain disorders in animals and humans. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer, and it is this aspect that has evoked global concern over feed and food safety, especially for milk and milk products[1]. Aflatoxin M_1 (AFM_1) is a hepatocarcinogen found in the milk of the animals that have consumed feeds contaminated with aflatoxin B_1 (AFB_1), the main metabolite produced by the fungi of the genus Aspergillus, particularly Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius[2]. About 0.3–6.2% of AFB_1 in animal feed is transformed to AFM_1 in milk[3]. Since AFM_1 has been evaluated as a possible human carcinogen, the cancer risk arising from AFM_1 contamination in milk is a serious problem in food safety[4]. The occurrence of AFM_1 in milk, especially cow's milk, makes it a particular risk factor for humans because of its importance as a foodstuff for adults and especially for children[5]. Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country[6]. The European Community prescribes that the maximum level of AFM_1 in liquid milk should not exceed 0.05 mg/kg. However, according to the US standard, the level of AFM_1 in liquid milk should not be higher than 0.5 mg/kg[7]. The objective of this study was to evaluate the occurrence of AFM_1 using ELISA method in milk distributed in Mashhad.

MATERIALS AND METHODS

Materials

Samples

In this study, the levels of AFM_1 in raw milk samples

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in Mashad-Iran were determined in June 2013. A total of 61 milk samples (1000 mL milk sample) collected by simple random sampling method. The samples were transported to the laboratory in an insulated container at about 4_Ć and analyzed upon arrival.

Reagents

Most of the reagents used to detect AFM_1 were contained in the RIDASCREEN test kit, which included microtiter plate coated with capture antibodies, AFM_1 standard solutions used for the construction of the calibration curve (1.3mL each 0, 5, 10, 20, 40, 80 and 200ppt), peroxidase conjugated AFM_1, substrate (urea peroxidase), chromogen (tetramethylbenzidine), and stop reagent contains 1N sulphuric acid. Methanol used was of analytical grade and provided by Merck.

Methods

AFM_1 detection

The quantitative analysis of AFM_1 in pasteurized milk samples was performed by competitive ELISA (RIDASCREEN AFM_1, R-Biopharm) procedure as described by R-biopharm GmbH[8]. Prior to analysis of the samples, the ELISA method was validated to ensure data quality. Validation of ELISA was carried out by determination of recoveries and the mean variation coefficient for fresh milk spiked with different concentrations of AFM_1 (5, 10, 20, 40, 80 and 200ppt). The results are expressed in TABLE 1.

Milk samples were centrifuged at 3500 g for 10 min at 10°C. The upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted phase) 100 μL was directly used per well in the test. One hundred μL of the AFM_1 standard solutions (100 μL/well) and test samples (100 μL/well) in duplicate were added to the wells of microtiter plate and incubated for 60min at room temperature in the dark. After the washing steps, 100 μL of the enzyme conjugate was added and incubated for 60min at room temperature in the dark. The washing step was repeated three times. Fifty μL of substrate and 50 μL of chromogen were added to each well and mixed thoroughly and incubated for 30min in the dark.

Following the addition of 100 μL of the stop reagent to each well, the absorbance was measured at 450 nm in ELISA reader (ELX-800, Bio-Tek Instruments, USA). According to the RIDASCREEN kit guidelines, the lower detection limit is 5 ppt for milk.

Evaluation of AFM_1

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standards) and multiplied by 100 (percentage maximum absorbance). Therefore, the zero standard is thus made equal to 100%, and the absorbance values are quoted in percentages. The values calculated for the standards were entered in a systerm of coordinates on semilogarithmic graph paper against the AFM_1 concentration in ppt.

Statistical analysis

The results were analyzed by Excel 2007 software and results presented as mean±SD.

RESULTS AND DISCUSSION

The standard solutions of concentrations of 0, 5, 10, 20, 40, 80 and 200ppt AFM_1 were used to find calibration/standard curve. Figure 1 gives the calibration curve of standard solutions of AFM_1 with concentrations of AFM_1 by ELISA analysis.

Analytical results showed that the incidence of

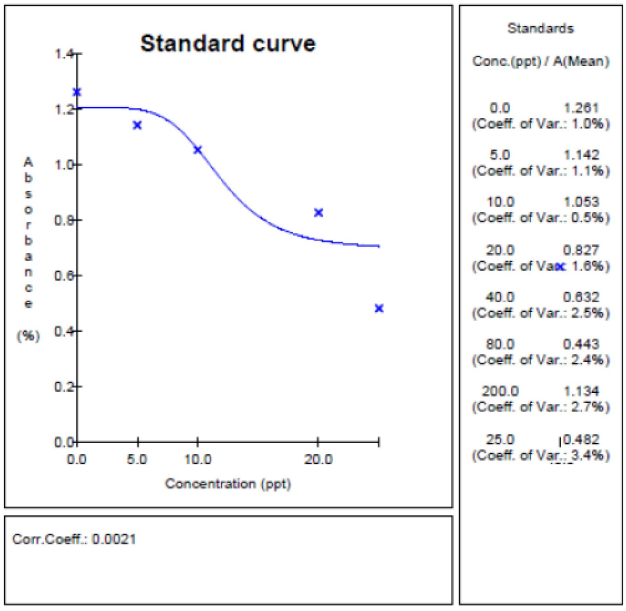

Figure 1 : Calibration curve of standard solutions of AFM_1 with concentrations of 0, 5, 10, 20, 40, 80 and 200ppt by ELISA method

AFM_1 contamination in raw milk samples was relatively high. AFM_1 was found in 53 (86%) of the examined milk samples by average concentration of 118.6ng/L

and the contamination level ranging between 0 and 250ng/L. The concentration of AFM_1 in 30 (49%) samples was lower than the Iranian national standard (100ng/L) but the mycotoxin level in all the samples was lower than Food and Drug Administration limit (500 ng/L), and only in 9 (14.7%) of the samples, the concentration of AFM_1 was greater than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission.

The mean concentrations of AFM_1 in milk from European, Latin American and Far Eastern diets have been reported by the Joint FAO/WHO Expert Committee on Food Additives[9], to be 23, 22 and 360 ng/L, respectively. Thus, the observed mean concentration of AFM_1 in Mashad milk samples was higher than the European and Latin American and lower than those reported in the Far Eastern diets. Also according to TABLE 1, the incidence of AFM_1 observed in the present study was higher than the incidence of AFM_1 reported by other authors[10-18]. The variations may be attributed to the differences in the regions, the shape of animal feeds[19,20], season and 4 special analytical methods. We suggest more researches on determination of this mycotoxin in dairy products to have a strict situation on the contamination.

TABLE 1 : The incidence of milk contamination in other studies

Location	Sample size	Percent of contamination	Percent of Contamination >50ng/L
Brazil (Sao Paulo)	125	95.2	26.4
India (Lucknow)	87	87.3	99
Morocco (Rabat)	54	88.8	7.4
Pakistan (Punjab)	168	100	99.4
Syria	126	80	52
Turkey (Anatolia)	129	58.1	47
Iran (Ahwaz)	311	42.1	12.5
Iran (Sarab)	111	76.6	40
Iran (Shiraz)	624	100	17.8
Iran (Tehran)	210	55.2	33.3
Average	194.5	78.33	43.48

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