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Determination of AFM1 in milk by ELISA technique in quchan (North-east of Iran)

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ABSTRACT

The aim of this study was to detect the amount of aflatoxin M1 (AFM1) in raw milk samples in Quchan in northeast of Iran. For this purpose, 34 milk samples were collected from Quchan milk collection centers during November 2012 and analyzed for AFM1 by enzyme linked immune-sorbent assay (ELISA) technique. All the analyses were done twice. Results showed presence of AFM1 in 88.23% of the examined milk samples by average concentration of 65.04ppt and contamination level ranging between 5.33 and 248 ppt. The concentration of AFM1 in all the samples was lower than the Food and Drug Administration (FDA) limits (500ppt), and, in 13 (38.23%) samples, AFM1 concentration was more than the maximum tolerance limit (50ppt) accepted by European Union (EU) and Codex alimentarius commission (CAC), and in 10 (29.41%) samples, AFM1 concentration was more than the maximum tolerance limit accepted by Iranian national standard (100ppt)

KEYWORDS

Aflatoxin M1;
Milk;
ELISA;
Quchan.

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 which has evoked global concern over feed and food safety, especially for milk and milk products[1]. Aflatoxin M1 (AFM1) is a hepato-carcinogen found in milk of animals that have consumed feeds contaminated with aflatoxin B1 (AFB1), the main metabolite produced by fungi of the genus *Aspergillus,* particularly *A. flavus, A. parasiticus,* and *A. nomius*[2]. About 0.3–6.2% of AFB1 in animal feed is transformed to AFM1 in milk[3]. Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country[4]. The European Community prescribes that the maximum level of AFM1 in liquid milk should not exceed 50 ppt. However, according to the US standard, the level of AFM1 in liquid milk should not be higher than 500ppt[5]. There have been several studies on AFM1 concentration in milk samples in different regions of the world and also in Iran, but this study was done to evaluate the occurrence of AFM1 in raw milk in Quchan in northeast of Iran that no study has ever been done in this area.

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MATERIALS AND METHODS

Materials

In this study the AFM1 content of raw milk samples in Quchan (northeast of Iran) was determined in Nov 2012. Thirty-four raw milk samples from milk collection centers collected by simple random sampling method. The samples were transported to the laboratory in an insulated container at about 4æ%C and analyzed upon arrival.

Methods

Samples treatment

Cold milk samples centrifuged for 10 min at 2000 g at 4ºC. The upper fat layer removed using a spatula. 100µl portions of the defatted milk samples used in the ELISA kit test (Europroxima, Netherlands).

AFM1 detection

The quantitative analysis of AFM1 in pasteurized milk samples was performed by competitive ELISA (Europroxima, Netherlands) procedure. Milk samples were centrifuged at 2000 g for 10 min at 4æ%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 AFM1 standard solutions (0, 6.25, 12.5, 25, 50, 100 and 200 pg/ml) 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 Europroxima kit guidelines, the lower detection limit is 5ppt for milk[6].

Statistical analysis

Data were analyzed using Excel 2007 and results reported as mean ± SD. The calibration curve and trendline equation prepared using Excel 2007.

RESULTS AND DISCUSSION

Interpretation of results of test

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[6]. The values calculated for the standards are plotted (on the Y-axis) versus the AFM1 equivalent concentration (pg/ml) on a logarithmic X-axis. The amount of AFM_1 in the samples is expressed as AFM_1 equivalents. The AFM_1 equivalents in the samples (ppt) corresponding to the jmaximal absorbance of each extract can be read from the calibration curve (Figure 1). The equation of the trendline in Figure 1 is as follows:

$$y = -0.330\,x + 75.97$$

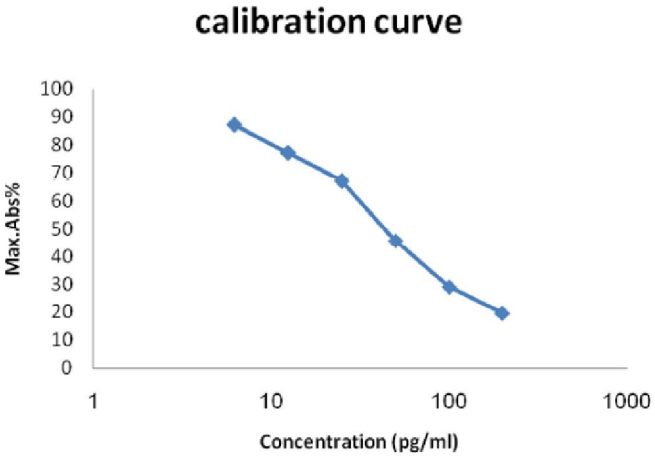

Figure 1: Calibration curve of standard solutions of AFM_1 with concentrations 6.25, 12.5, 25, 50,100, and 200ppt by ELISA analysis.

TABLE 1 : AFM_1 distribution in raw milk samples.

AFM_1 levels (ppt)	<10	10-<50	50-100	>100
Number of contaminated samples	9	12	3	10
Percentage of samples containing AFM_1	26.47	35.29	8.82	29.41

Results showed presence of AFM1 in 88.23% of the examined milk samples by average concentration of 65.04ppt and contamination level ranging between 5.33 and 248 ppt. The concentration of AFM1 in all the samples was lower than the Food and Drug Administration limits (500ppt), and, in 13

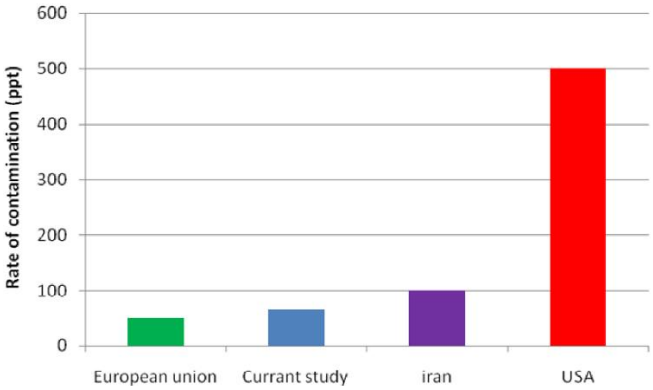

Figure 2: Comparing the results with maximum tolerance limit in some countries

(38.23%) samples, AFM1 concentration was more than the maximum tolerance limit accepted by European Union and Codex Alimentarius Commission (50ppt), and in 10 (29.41%) samples, AFM1 concentration was more than the maximum tolerance limit accepted by Iranian national standard (100ppt) (Figure 2).

The mean AFM1 concentrations in milk in European, Latin American, and Far Eastern diets have been reported by the Joint FAO/WHO Expert Committee on Food Additives[18] to be 23, 22, and 360ng/L, respectively. Thus, the observed mean AFM1 concentration in Quchan milk samples was as high as the European and Latin American and much lower than those reported for the Far Eastern diets. On the other hand, several studies have been done to determine AFM1 contamination of milk in Iran (TABLE 2). In all studies, the averages of toxin concentrations are below 100 ppt. The variations may be attributed to differences in region, season, and especially analysis method.

TABLE 2 : The incidence of milk contamination in Iran in other studies

Location	Reference	Method of detection	Sample size	Percent of contamination	Percent of contamination >50 ppt	AFM1 concentration (ppt)
Quchan	Current study	ELISA	34	88.23	38.23	65.04
Mashad (north east of Iran)	Mohamadi Sani et al.,2012[7]	ELISA	42	97.6	9.8	23.2
Mashad (north east of Iran)	Mohamad Sani and Nikpooyan, 2012[8]	HPLC	60	100	1.6	16.16
Mashad (north east of Iran)	Mohamadi Sani et al., 2010[9]	ELISA	196	100	80.6	77.9
Five states of Iran	Tajkarimi et al., 2007[10]	HPLC	98	100	37.7	39
Tehran (capital of Iran)	Heshmati and Milani, 2010[11]	ELISA	210	55.2	33.3	58
14 states of Iran	Tajkarimi et al., 2008[12]	HPLC	319	54	23	57
Shiraz (south of Iran)	Alborzi et al., 2006[13]	ELISA	624	100	17.8	Not reported
Ahwaz (south of Iran)	Rahimi et al., 2010[14]	ELISA	311	42.1	12.5	43.3
Sarab (north west of Iran)	Kamkar 2005[15]	TLC	111	76.6	40	61.4
Central part of Iran	Fallah 2010[16]	ELISA	225	67.1	33.1	49.9
Ardabil (north west of Iran)	Nemati et al., 2010[17]	ELISA	90	100	33	Not reported

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