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INCIDENCE OF AFLATOXIN M1 IN FRESH MILK MARKETED IN QUETTA, PAKISTAN

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Abstract:

Introduction: *The occurrence of Aflatoxin M1 (AFM1) in milk is concern to public health because it is classified as toxic compound in group I by the International Agency for Research on Cancer (IARC). AFM1 is a hepatocarcinogen. Additionally, it can cause DNA damage, gene mutation, chromosomal anomalies, immune-suppression and cell transformation. It can be present in milk of animals that have used feed infected with aflatoxin B1 (AFB1) which is the main metabolite produced by Aspergillus fungal species such as, particularly A. flavus, A. parasiticius and A. nomius. This toxin is produced by fungus on different substrate (food, edible raw materials, and feed). About 0.3 to 6.2% of Aflatoxin B1 (AFB1) toxin in feed is altered to AFM1 in milk. Presences of aflatoxins in food cause public health concern and the consequent economic lose. Keeping in view the above facts the present study was designed to determine the AFM1 in milk marketed in Quetta, Pakistan.*

Materials and methodology: *The study was conducted in Quetta city to quantify the milk toxin. The total 100 milk samples were collected from different lacalities of Quetta city. AFM1 in milk samples was quantified with the help of Enzyme Linked Immunosorbent Assay (ELISA).*

Results*: Incidence of high percentage of contaminated milk samples with AFM1 was found. The mean % (ng/l) of milk toxin AFM1 in all samples was greater than the tolerance limit accepted by European Community (50ng/l) and below than United States (US) regulations (500ng/l). The lowest and highest mean % of AFM1 in milk was 236.63ng/l and 292.96ng/l respectively. The higher values might be due to heavily contamination of animal feed with AFB1 and poor hygienic conditions.*

Key Words: *AFM1, quantitative, milk, ELISA, market, Quetta*

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INTRODUCTION:

In today's era genuineness and safety of food is more vital because of great dependability of the people on the food industry to supply best food for use. The occurrence of aflatoxin M1 (AFM1) in milk is concern to public health because it is classified as toxic compound in group II by the IARC [1]. However, after additional studies IARC has been reclassified AFM1 as Group 1 carcinogen [2]. As the AFM1 having carcinogenicity 2-10% higher than its original form AFB1, furthermore it can cause gene mutation, DNA damage, chromosomal anomalies, cell transformation and immune-suppression along with AFB2 and G1 [3, 4]. The milk toxin AFM1 is a mycotoxin. Mycotoxin produces as secondary metabolite by molds. They are highly toxic and cause certain types of cancer in animals and human. This feature has evoked worldwide concern for milk and its products safety [5].

AFM1 is a hepatocarcinogen. It can be present in milk of animals that have used feed infected with aflatoxin B1 (AFB1). AFB1 is a toxin which is the main metabolite produced by fungi *Aspergillus*, particularly *A. flavus*, *A. parasiticius* and *A. nomius* [6]. This toxin is produced by fungus on different substrate (food, edible raw materials, and feed). About 0.3 to 6.2% of AFB1 toxin in feed is altered to AFM1 in milk [7]. The aflatoxins ingested by the animal absorbed in the gastrointestinal tract and bio transformed in liver, with the help of microsomal enzymes related to cytochrome P450 [8] in various forms, among them hydroxylation and epoxidation stand out. Hydroxylation process form derivative such as aflatoxin M1, which excreted in milk of animal [9].

Aflatoxin B1 is the most toxic than B2, G1 and G2 [10]. IARC recommended it as probable human carcinogens of group II B and AFB1 has been recommended as a carcinogen of group1 [1]. The main substances of aflatoxin family are aflatoxin, G1, G2, B1 and B2. Now a day, more than ten compounds are included in family of aflatoxin, and they are derivatives of the four main substances. The European community set 0.05ug/kg the maximum level of AFM1, in milk [11]. Aflatoxins are hardly decomposed during cooking of contaminated food. The occurrence of AFM1 in milk is a matter of concern as it provides main nutrients for people of different age groups.

Presences of aflatoxins in food cause public health concern and the consequent economic lose. Keeping in view the above facts the present study was designed to determine the AFM1 in milk marketed in Quetta, Pakistan. Different analytical techniques were used for determination of AFM1 in milk like Capillary electrophoresis, High performance Liquid Chromatography (HPLC), Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). However, in the present study, ELISA method was used because it is faster, reliable and inexpensive technique for the detection of toxin in milk.

MATERIAL AND METHODS:

Sampling

The milk samples were collected from different ten localities such as Brewery road (L1), Satellite town (L2), Jail road (L3), Huda and Deba (L4), Sabzal road (L5), Arbab Karam Khan road (L6), Quary road (L7), Sirki road (L8), Kasi road (L9), Alamdar road and Cantonment (L10). From each locality, ten samples were randomly collected. Thus the total samples of milk were 100.

Experiment

AFM1 in milk samples was quantified with ELISA kit (Agra Quant aflatoxin M1 sensitive, item no COKAQ 7100, Quantitation range 25-500ppt and detection limit 18ppt). Kit contained micro well titration plate. The Milk samples were centrifuged and skimmed.100ul of skimmed milk samples and given standard solution of AFM1 per well was used. Milk samples and standard solution were added in to each well (the wells were already coated with anti-aflatoxin M1 antibodies by the manufacture). Incubation was carried out for 60 minuets at room temperature. The micro titer plates were washed four times by washing buffer. After washing step enzymes conjugate solution (100ul) was added in plates and incubated again as previously. To remove unbound enzyme conjugate well was washed as before and 100ul developing solution was added in samples and standards. Now incubation was carried in a dark place for 15 minutes at room temperature. Stop solution was added to stop reaction. ELISA reader (Bio Tek, ELx800) was used to determine the absorbance at 450nm within 60 minutes. The calculation was made as follows:

$$\%\text{Absorbance (B/B0)} = \frac{\text{Absorbance of standard or sample (B)}}{\text{Absorbance of Zero standards (B0)}} \times 100$$

The standard curve (Fig. 1**)** was constructed by plotting the % absorbance (relative absorbance %) of the standards and corresponding concentration of AFM1 in ppt. AFM1 standard solutions of 0, 25, 50, 100, 200 and 500ppt were used for the standard curve. The concentrations of AFM1 in milk samples were determined by plotting % absorbance values of milk samples in standard curve. Data were analyzed through applying regression by using Excel 2007. Linear standard curve was obtained over the range studied.

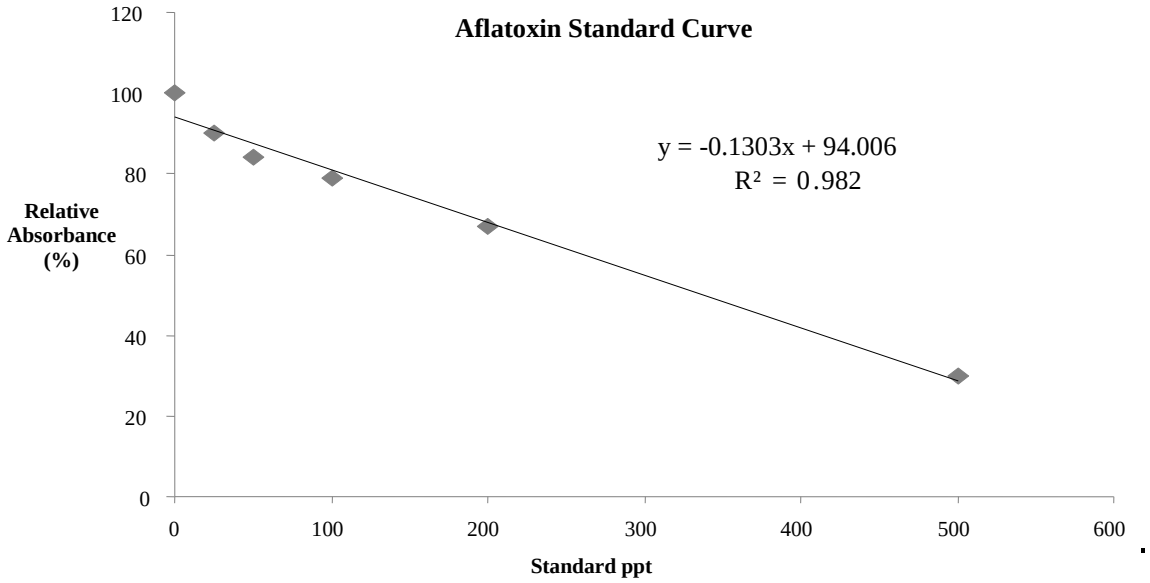

Fig. 1: ***Calibration curve of standard solutions of AFM1***

RESULTS AND DISCUSSION:

Analytical results showed that 90% milk samples from locations L1, L3, L8, L10, 80% milk samples from locations L2, L5 to L7 and 100% of milk samples collected from location L4 and L9 were contaminated with milk toxin as represented in Table 1. Incidence of high percentage of contaminated milk samples with AFM1 was found.

Table 1: Incidence of AFM1 contaminated milk samples in different localities of Quetta city

Sources of milk samples (n=10)	Positive samples (%)	Minimum (ppt)	Maximum (ppt)	Mean (ppt)	±SD
L1	9 (90)	40.71	437.41	261.53	130.56
L2	8 (80)	47.62	406.72	281.74	150.46
L3	9 (90)	44.55	475.77	236.63	141.63
L4	10 (100)	20.00	422.06	238.60	125.49
L5	8 (80)	47.62	345.33	240.50	130.81
L6	8 (80)	196.48	468.10	292.96	143.0
L7	8 (80)	47.62	422.06	255.56	143.3
L8	9 (90)	38.41	468.10	241.60	146.7
L9	10 (100)	19.23	422.06	264.38	132.4
L10	9 (90)	49.15	376.02	260.84	121.4

The results revealed that milk samples collected from locations L1 to L10 have 261.53, 281.74, 236.63, 238.60, 240.50, 292.96, 255.56, 241.60, 264.38 and 260.84ng/l or ppt mean % concentration of AFM1 respectively. Statistically the mean percentages of AFM1 of milk samples were found non-significantly different (P>0.05).

The contamination of milk is considered as one of the serious issue in the last few years. Milk is an excellent source of nutrients. However milk could be a source of toxic substances such as AFM1. Aflatoxin M1 is hepatotoxic and carcinogenic. This toxin enters in to food chain and accumulates in the body of the end consumers. Due to health risk different countries have set tolerance limit of aflatoxin M1 in milk. The US regulation has set 500ng/kg of AFM1 in milk [12]. While European Community accepted limit of AFM1 in milk is 50ng/kg [13].

The results further revealed that the mean % (ng/l) of milk toxin AFM1 in all samples were greater than the tolerance limit accepted by European Community (50ng/l) and below than US regulation (500ng/l). The lowest and highest mean % of AFM1 in milk was 236.63ng/l and 292.96ng/l respectively. The higher values might be due to heavily contamination of feed of animal by aflatoxin B1, because there is strong correlation between the milk toxin AFM1 and AFB1. AFB1 converts in to AFM1. The level of AFB1 in feed depends on temperature and moisture. The feed having 13-18% water contents are the best source for the growth of toxin producing fungi *Aspergillu. flavus and Aspergillus. parasiticus*.

Several studies have been reported the high levels of toxin in milk. However the variation of results might be due to many factors like differences in climatic conditions, area and also the method used for analysis. In other study conducted in Faisalabad city, Pakistan, found low level of AFM1 in milk than European community limit 50ng/kg by using the ELISA technique [14] while high level of AFM1 in milk than European tolerance limit 50ng/kg was found in another study carried out in Lahore city, Pakistan by using HPLC with fluorescence detector [15]. Another study conducted in Iran which revealed that 98 milk samples were found positive for AFM1 and with mean concentration of 0.053ug/L. The study further revealed the occurrence of higher level of toxin in winter and spring than in summer and autumn [16].

CONCLUSIONS:

It is concluded that the mean % (ng/l) of milk toxin AFM1 in all samples was greater than the 50ng/l limit prescribe by European Community and below than 500ng/l, limit set by the US regulation. The higher values might be due to heavily contamination of animal feed with AFB1, because there is strong correlation between milk toxin and the main metabolite AFB1 produced by fungus.

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