International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 01 January 2024

Page No: 30-36

DOI: https://doi.org/10.47191/ijpbms/v4-i1-05, Impact Factor: 6.858

Detection of Aflatoxin M1 and Several Heavy Metals in Medical Infant Milk Formula Sold in Iraqi Markets

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ABSTRACT

Milk is essential for infants, and infant feeding should be emphasized in any program to ensure good child health. To find harmful and trace elements in different milk powder brands bought in Iraq and fed to babies with metabolic syndrome, atomic absorption spectrometry (AAS) was used. Fluorescence high-performance liquid chromatography (HPLC) was also used to measure aflatoxin M1. The result reveals the concentrations of elements close to the product label's description, such as magnesium, calcium, zinc, selenium, iron, and manganese. Only the potassium element was higher than what was listed on the product label. Additionally, no quantities of hazardous elements like lead (Pb), cadmium (Cd), or arsenic (As) were found. As for aflatoxin M1, it was found that the PKU milk formula and lactose-free milk formula were found to be free of AFM1 contamination, indicating that they are safe to consume. AFM1 was found in concentrations above the permissible limit in the MSUD milk formula (0.08 µg/kg). The reason for this issue could be inadequate storage. The other two types, TYR and OAc milk formula, were lower than the allowable limit of 0.05 µg/kg. As a result, there needs to be more oversight of the milk that newborns with metabolic syndrome consumele S. aureus or Streptococcus pyogenes, and Streptococcus pneumoniae in community settings.

KEYWORDS: Aflatoxin M1, heavy metals, infant milk formula, metabolic syndrome.

ARTICLE DETAILS

Published On:
16 January 2024

Available on:
https://ijpbms.com/

INTRODUCTION

Since milk is a vital source of proteins, lipids, vitamin supplements, and essential minerals, it is considered a virtually complete food[16]. Milk is essential for infants; infant feeding should be emphasized in any program to ensure good child health. Certain circumstances may necessitate a diet for metabolic reasons, or artificial feeding may be required. Raw milk from cows or buffalo is a natural alternative to human milk for baby feeding. Powdered milk is made from concentrated liquid milk that has been dried[31].

Milk is a nutrient-dense food high in micro and macronutrients essential for human health and growth. As a result, milk's safety and hygiene are crucial for health[8]. The escalation of pollution levels has worsened the problem of milk contamination, leading to heightened apprehensions regarding milk quality[14]. The presence of environmental contaminants and xenobiotic compounds in bovine feed, such as toxic metals, mycotoxin, dioxin, and other pollutants, is believed to have a significant impact on public health globally through the contamination of milk. According to reports, approximately 38 micro and trace elements are present in raw milk from various places throughout the world[20].

Twenty amino acids are needed for metabolism; humans cannot produce nine, and they must be obtained through diet. Genetic issues with one or more amino acids' metabolism are the leading cause of most diseases related to amino acids. Several examples are phenylketonuria (PKU), maple syrup urine disease (MSUD), classical homocystinuria (HCU), tyrosinemia (TYR), cystathionine-β-synthase insufficiency, and more[32].

Aflatoxin M1 is a hydroxylated metabolite of AFB1 that is deformed inside the liver through cytochrome P-450 enzymes[26]. AFB1 secretes this, which is absorbed through food, through the milk glands of breastfeeding cows[25], and approximately 0.5% to 6% of AFB1 is transformed into AFM1. When AFB1 polluted animal diets, it caused lactating animals to become diseased., AFM1 was identified between 12 and 24 hours and peaked after 72 hours when the animals were fed the contaminated animal feeds[23]. Several kinds of research have been carried out around the world to see how common AFM1 is in both industrial and traditional milk; for instance, Jafari[21] conducted research in Tiran County, Isfahan Province, Iran, revealing that AFM1 concentration in industrial milk (15 brands) was measured; the results revealed that nearly 45 per cent of industrial milk samples were contaminated with AFM1 above the E.U. and Codex standard levels. Metal and other toxic food poisonings are among the most critical problems in developing countries. There have been several studies undertaken throughout the world about health dangers, for instance; In Pakistani markets, Aflatoxin was examined in Infant Milk Formula products[2], as well in Iran[9], and in Egypt1, and many other similar studies. In 1990, the United States enacted the Nutrition Labelling and Education Act (NLEA); following this law, the FDA was given the power to require that the Nutrition Facts label be put on either the side or back of a food box; this label tells you how much cholesterol, salt, carbs, and protein are in a product[10].

Given the preceding concerns, this study is critical regarding Iraq's public health risks. This study aimed to look for aflatoxin M1 and several heavy metals in various newborn milk formula brands available in Iraqi markets used by metabolic syndrome babies to see if their intakes are within the recommended levels.

MATERIALS AND METHODS

Sample collection

The study is focused on the milk consumed by infants with metabolic syndrome; therefore, five brands of milk with three replications of each one were purchased from local markets and supermarkets (15 samples; only these types are available in Iraq for these diseases or disorders); for the collected samples, specific manufacturing and expiration dates were recorded; and it was five different types (PKU milk formula, Lactose-Free milk formula, MSUD milk formula, OAc milk formula, and TYR milk formula). All samples were prepared in aseptic circumstances using sterilized materials for subsequent analysis.

Estimation of Trace Elements and Heavy Metals

Flame and flameless atomic absorption spectrometry were applied to determine Mg, Ca, Zn, Se, Fe, K, Mn as trace elements and b, Cd, As) as toxic heavy metals in Infant Formula Milk Brands. According to the acid digestion[6], 3 g of the sample was placed into a Teflon beaker (three replicates), and 3 ml of concentrated perchloric acid solution was covered. Then, it was heated gently on an electric hot plate and increased temperature to complete the digestion process; when the mixture reached the dryness stage, we removed the cup from the heat and added another 3 ml of the concentrated nitric acid solution, covered the cup, and was heated and evaporated samples until approached the drying stage, then 5 ml of diluted hydrochloric acid solution was added with distilled water in a ratio of (1:1) after filtered through 0.45 µm Millipore filters as the sample became ready for analysis. Using the Atomic Absorption instrument, the absorbance of these digested samples was determined[6].

HPLC Chemical Analysis for Aflatoxin M1 Residues

Qualitative and quantitative Infant Formula Milk Brands analyses were done using High-Performance Liquid Chromatography HPLC/ESI-MS. Sonicated 25 g of sample for 40 minutes in 100 mL 70:30 v/v methanol: water, after being centrifuged for 5 minutes, 5 ml of the resulting liquid was extracted. This 5 mL was mixed with 20 mL of water to create a diluted solution. The diluted solution was then passed through the immunoaffinity column at 3 mL per minute. Before this, the column was prepared by being conditioned with 20 mL of distilled water. HPLC machine was conditioned by pumping mobile phase solution at a steady flow rate till a stable baseline developed. To design the calibration curve, working standard solutions at various concentrations of AFM1 g/L in the mobile phase were produced[5].

The analysis was conducted in the Department of Environment and Water/ Ministry of Science and Technology, Iraq. The following equation determined the concentration of the isolated compound:

$$\text{The concentration of sample}\left(\frac{\mu g}{ml}\right) = \left\{\frac{\textbf{Area of the sample}}{\textbf{Area of the standard}}\right\} \times \textbf{Standard con.} \times \textbf{Dilution factor}$$

Statistical Analysis:

Milk is essential for infants; infant feeding should be emphasized in any program to ensure good child health. Atomic Absorption Spectrometry (AAS) was used to detect hazardously and trace elements in different brands of milk powder purchased from the Iraqi market and consumed by infants with metabolic syndrome, as well as aflatoxin M1 was measured utilizing fluorescence high-performance liquid chromatography (HPLC). The result reveals the concentrations of elements close to the product label's description, such as Magnesium Mg, Calcium Ca, Zink Zn, Selenium Se, Iron Fe, and Mn Manganese. Only the potassium K element was higher than what was listed on the product label. Additionally, no quantities of hazardous elements like lead (Pb), cadmium (Cd), or arsenic (As) were found. As for aflatoxin M1, it was found that the PKU milk formula and Lactose-Free milk formula were found to be free of AFM1 contamination, indicating that they are safe to consume. AFM1 was found in concentrations above the permissible limit in MSUD milk formula (0.08 µg/kg). It may be due to poor storage, while the other two types, TYR and OAc milk formula, were lower than the allowable limit of 0.05 µg/kg. Therefore, the milk consumed by newborns with metabolic syndrome needs more monitoring. The Statistical Analysis System[27] application was utilized. In this study, the least significant difference (LSD) test (ANOVA) was utilized to compare means.

RESULTS AND DISCUSSION

The milk must be monitored as one of the potential sources of heavy metals because early-life exposure might produce alterations that manifest much later in life[28]. Concerns about the effects of Pb, Cd, As, and other hazardous metals on public health have developed due to their accumulation in the environment, particularly in agricultural and livestock production, expanding the possibility for pollutants to enter human consumption[24]. Analysis of milk samples indicated there was no contamination with metalloids such as lead, cadmium, and arsenic, as in Table 2. As a result, the absence of hazardous metals in all samples of this research demonstrates the safety of their consumption by newborns.

Milk and its products are the primary dietary sources of calcium (Ca), along with potassium (K), magnesium (Mg), and phosphorous (P), which are the most prevalent elements[29]. It also has around twenty different trace elements, most of which are essential and necessary, like copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe)[18]. These metals interact with enzymes to fulfil a crucial purpose in numerous cellular processes[12]. Table 1 summarizes the results; the findings show that the concentration of these elements Mg, Ca, Zn, Se, Fe, K, and Mn differs between what is present in the samples and what is listed on the product labels, but at concentrations that are safe for the infant who consumes this product. Some minerals, such as Fe and Zn, were significantly ($P \leq 0.01$) decreased compared with levels listed on the product label (6.00 ±0.00 and 5.30 ±0.00) mg/100g, respectively. According to the findings, potassium concentrations in all milk brands were significantly ($P \leq 0.01$) increased compared with levels listed on the product label 474.00 ±0.00 mg/100g, in addition to a significant ($P \leq 0.01$) increase of Mg in PKU samples 70.66 ±0.54 mg/100g, and a significant ($P \leq 0.01$) decrease in MSUD and OAc samples (52.06 ±4.29, 52.59 ±0.88) mg/100g respectively, compared with the listed on the product label 63.00 ±0.00 mg/100g. The mismatch between the stated quantity of the required constituents (energy compounds, vitamins, or critical elements) and the actual amount frequently arises due to what is known as "overages". The concentration of ingredients in food products and dietary supplements increases proportionally when the quantity of these products diminishes over time during storage[34]. The products are formulated in such a way that the quantity of content does not fall below the declared amount by the end of the expiration date. However, although these production practices are generally acknowledged, inconsistencies might result in physiological and metabolic disruptions in the body. Furthermore, it might further complicate the evaluation of daily nutrient consumption[33]. Also, the results revealed a significant ($P \leq 0.01$) increase in Ca in PKU and Lactose-Free milk (593.82 ±8.92, 552.31 ±17.81 mg/100g respectively and significantly decreased in Ca in TYRo 454.12 ±15.20 mg/100g compared with the amount specified on the product label 496.00 ±0.00 mg/100g, on the other hand, When compared to the amount specified on the product label, all samples had a significant decrease in Zn. Furthermore, as compared to the amount specified on the product label, all samples show a significant rise in Se, all samples revealed a significant decrease in Fe compared with the amount specified on the product label, the assessment of K level showed a significant increase in all samples except the OAc sample 500.47 ±10.05 mg/100g which show non-significant compared with the amount specified on the product label 474.00 ±0.00 mg/100g. Finally, the results show a significant increase in Mn level in PUK sample 0.523 ±0.06 mg/100g compared with the amount specified on the product label 0.399 ±0.00 mg/100g. In contrast, other samples have shown non-significant compared with the amount specified on the product label.

Table 1: Comparison between difference groups in Trace elements in infants milk formula mg/100g

Group	Mean ± SE						
	Mg	Ca	Zn	Se	Fe	K	Mn
PKU milk formula	70.66 ±0.54 a	593.82 ±8.92 a	3.75 ±0.13 c	0.048 ±0.002 a	4.89 ±0.11 bc	812.13 ±11.41 b	0.523 ±0.06 a
Lactose-Free milk formula	57.75 ±1.39 bc	552.31 ±17.81 b	3.25 ±0.14 cd	0.0366 ±0.002 b	3.94 ±0.11 de	757.78 ±14.92 bc	0.442 ±0.02 ab
MSUD milk formula	52.06 ±4.29 d	489.41 ±2.50 c	2.86 ±0.31 d	0.0260±0.005 c	4.49 ±0.52 cd	714.76 ±0.23 c	0.411 ±0.007 b
OAc milk formula	52.59 ±0.88 cd	515.68 ±13.02 c	3.63 ±0.18 c	0.0296 ±0.004 bc	3.60 ±0.17 e	500.47 ±10.05 d	0.421 ±0.012 b

TYRo milk formula	60.22 ±0.27 b	454.12 ±15.20 d	4.64 ±0.29 b	0.0292 ±0.005 bc	5.23 ±0.19 b	962.19 ±15.05 a	0.424 ±0.011 b
(Listed on the product label)	63.00 ±0.00 b	496.00 ±0.00 c	5.30 ±0.00 a	0.015 ±0.00 d	6.00 ±0.00 a	474.00 ±0.00 d	0.399 ±0.00 b
LSD value	5.53 **	33.79 **	0.601 **	0.0106 **	0.715 **	67.45 **	0.083 *
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0492
Means in the same column with different letters differed significantly. * (P≤0.05), ** (P≤0.01).							

As for mycotoxins, some Aspergillus species produce aflatoxins, which are poisonous, carcinogenic, and immunosuppressive secondary metabolites. It is biotransformed from AFB1 into AFM1 in the liver after 12-24 hours and then excreted in breastfeeding mammals' milk. The existence of AFM1 in milk is a cause for concern worldwide, as even low levels of this metabolite would significantly impact long-term exposure[17]. In milk and milk products, the European Commission specifies a maximum allowable level of 0.05 µg/kg AFM1[13]. Because AFM1 is heat stable (120°C) and cannot be removed or destroyed by chemical or physical treatments, the only effective way to prevent AFM1 contamination in milk and, consequently, infant milk formula is to monitor AFB1 levels in animal feeds[22]. For dairy animals such as cattle, sheep, and goats, the maximum permissible level of AFB1 incomplete meals has been determined at five µg/kg of AFB1[30]. As for the results of AFM1 contamination levels, the detection in milk powder in Table 2 shows a significant increase in AFM1 in MSUD (0.08 ±0.087 µg/kg; as in fig 1) while OAc milk formula (0.01 ±0.09; as in fig. 2) and TYR milk formula (0.011 ±0.34; as in fig 3) shows a significant decrease compared with permissible limit 0.05 µg/kg. AFM1 contamination levels in PKU and Lactose-Free milk formula were Nil (AFM1 does not exist in these samples Table 2). The Limit Of Detection (LOD) and Limit Of Quantification (LOQ) for AFM1 were 0.001–0.1 µg/kg and 0.01−0.085 µg/kg, respectively.

Table 2: Comparison between difference groups Aflatoxin M1 µg/kg

Group	Mean ± SE
PKU milk formula	0.001 ≈ Nil
Lactose-Free milk formula	0.001 ≈ Nil
MSUD milk formula	0.08 ±0.087 a
OAc milk formula	0.01 ±0.09 c
TYR milk formula	0.011 ±0.34 c
Permissible Limit	0.05 b
LSD value	0.021 **
P-value	0.0001
Means in the same column with different letters differed significantly. ** (P≤0.01).	

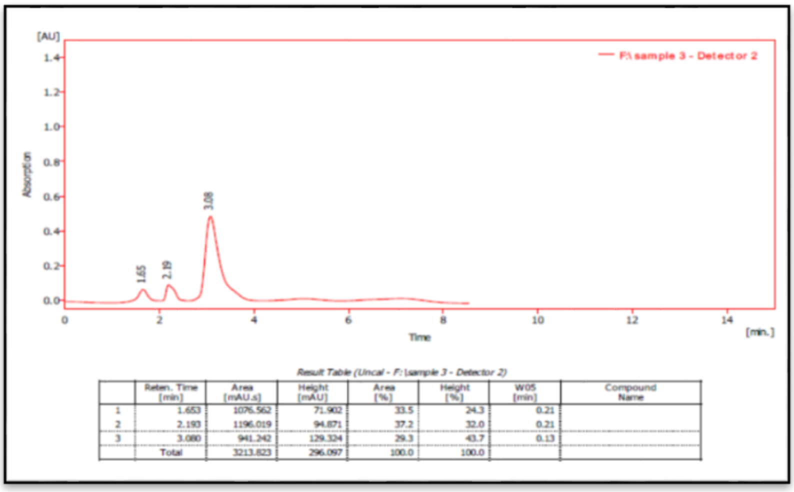
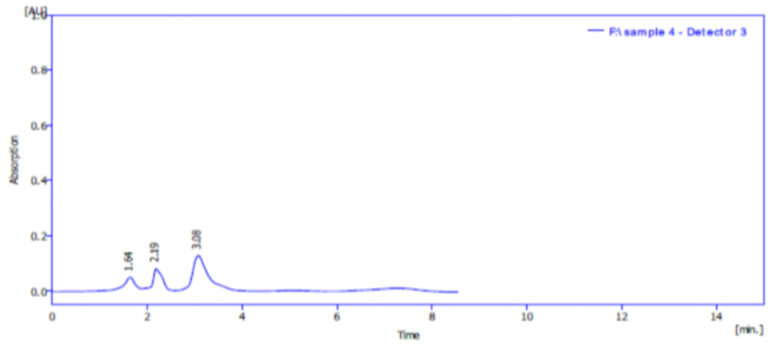


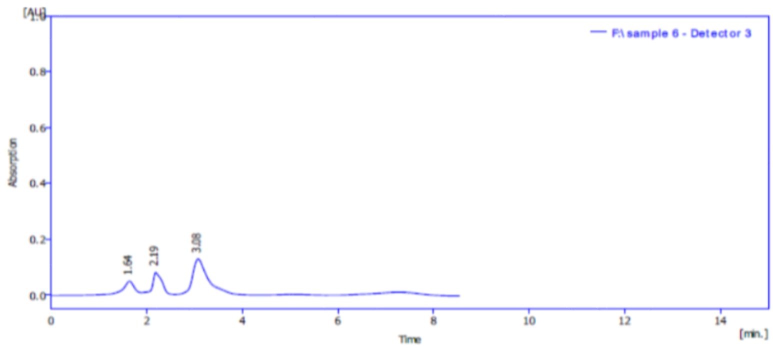
Figure 1: HPLC chromatograms of Aflatoxin M1 concentrations (0.08 µg/kg) in Maple Syrup Urine Disease milk formula that refer to the high accuracy of retention time.



Result Table (Uncal - F:\sample 4 - Detector 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.640	136.272	20.029	26.4	28.6	0.12	
2	2.190	136.799	16.138	26.5	23.1	0.07	
3	3.077	242.679	33.782	47.1	48.3	0.13	
	Total	515.749	69.949	100.0	100.0		

Figure 2: HPLC chromatograms of Aflatoxin M1 concentrations (0.01 µg/kg) in Organic Acidurias milk formula that refer to the high accuracy of retention time.



Result Table (Uncal - F:\sample 6 - Detector 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.640	136.272	20.029	20.8	20.7	0.12	
2	2.190	253.798	41.215	38.8	42.7	0.14	
3	3.077	264.126	35.356	40.4	36.6	0.14	
	Total	654.195	96.600	100.0	100.0		

Figure 3: HPLC chromatograms of Aflatoxin M1 concentrations (0.011 µg/kg) in Tyrosinemia milk formula that refer to the high accuracy of retention time.

In agreement with the results, the AFM1 level in the MSUD milk formula exceeded the E.U. maximum limit. The other two types, TYRo and OAc milk formula, were lower than the permissible limit but needed more monitoring. Additionally, Aspergillus and parasitic fungi, naturally present in food, produce poisons when exposed to optimal temperature conditions, relative humidity or moisture, and inadequate storage conditions[19], Aflatoxin may have been present due to poor storage. Because infants are especially vulnerable to the dangers of AFM1, adequate safeguards should be in place to ensure that all powdered milk brands sold in Iraq comply with the allowable limit. As a consequence, it is evident and critical that AFM1 contamination of infant milk formula is an issue worldwide and that protecting infants from AFM1 necessitates fundamental national and international cooperation as well as far-reaching revisions of national and international surveillance, toxicity, and threat assessment of AFM1 regulations and approaches[7]. Compared to the findings of other research, AL-Mossawei[3] showed that 40% (10 of 25 samples) of imported dry milk powder samples in Baghdad were contaminated with AFM1. Another study by Al-Sawaf[4] in Iraq on milk powder showed that 23 samples out of 29 were positive and higher than the permissible limits of AFM1. Additionally, AFM1 was discovered in 49 samples (53%) of newborn dry milk samples in an Italian investigation[15].

CONCLUSION

This is the first study in Iraq to assess the safety of this type of milk consumed by newborns with metabolic syndrome. It was found to be free of harmful metals, while the essential minerals were detected within, higher, or lower than listed on the product label at concentrations quite close. PKU and Lactose-Free milk were found to be free of AFM1 contamination, indicating that they are safe to consume. AFM1 was found in concentrations above the permissible limit in the MSUD milk formula. It may be due to poor storage, while the other two types, TYRo and OAc milk formula, were lower than the permissible limit.

ACKNOWLEDGEMENTS

The author would like to thank the Department of Environment and Water/Ministry of Science and Technology, Iraq, technicians for conducting all the analyses. Thank Dr. Salah Al-Chalabi for actively interpreting the statistical data.

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