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Aflatoxin B1 Contamination in Chicken Livers purchased from Karachi, Pakistan

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Abstract

Aflatoxins are toxic carcinogenic contaminants found in foodstuff as well as in feeds and are believed to be the primary health hazard. Determination of aflatoxins in food stuff and feeds is thus very important. Occurrence of Aflatoxin B1 in chicken liver samples purchased from various chicken slaughtering shops located at different areas of Karachi, Pakistan, was investigated. A total of 120 chicken liver samples were analyzed for contamination of aflatoxin. Occurrence of aflatoxin was detected for 49 (40.38%) out of 120 of the samples evaluated. 27 (38.57%) out of 70 samples of normal, 15 (42.85%) out of 35 samples of moderate and 7 (46.67%) out of 15 samples of pale colored chicken liver were found positive. Maximum level of aflatoxin B1 was detected as 5.02µg/kg for pale colored chicken liver and minimum level 2.38µg/kg in normal chicken liver. The findings of current study (5.02µg/kg maximum and 2.38µg/kg minimum level) are found to be lower than the prescribed standard limits of aflatoxin B1 10µg/kg in human food in Pakistan. The contamination of aflatoxin in chicken liver could be a serious problem for the largest populated city of Karachi, where limited resources are available for the prevention and controlling of their levels in the food supply.

Keywords: Aflatoxin, food safety, HPLC, chicken liver

Introduction

Commercial poultry production industry has become a dominant and productive sector of Pakistan contributing ≤13% to the GDP, and become major protein source provider to Pakistani population (Hussain, Rabbani *et al.* 2015). Chicken contributes 45% of the meat consumed in Pakistan. The consumption of poultry meat in Pakistani has been reported to be 9kg per capita, lesser then the per capita consumption of poultry meat by USA (48kg), Australia (44kg) and UAE (44kg). About 20000 poultry forms have been contributing 18,000 million eggs and 2,250 million kg chicken meat in Pakistan. 5215 poultry farms are contributing to provide fresh chicken meat and egg in Sindh out of which 585 poultry farms are located in Karachi city and its suburbs (Hussain, Rabbani *et al.* 2015, Sadiq 2004). In Pakistan, the cheapest protein source based on animal source is believed to be the chicken. The Pakistani population has been consuming 17gm of animal protein which is less than the daily requirement of 27gm set by World Health Organization (Memon 2012).

Aflatoxins are poisonous substances produced by two of the fungi, *Aspergillus parasiticus* and *Apergillus flavus*; they are responsible to contaminate food crops. This contamination of aflatoxin has become a serious health threat to humans. These moulds generally contaminate foodstuff under favorable conditions including high temperatures and high humidity particularly found in the equatorial and subtropical regions (Gourama and Bullerman 1995). Toxic effects due to contamination of aflatoxin such as liver cancer and lowering in immune response in various animals and humans have already been reported earlier (Williams, Phillips *et al.* 2004, Jiang, Jolly *et al.* 2005). Aflatoxin B1 (AFB1) is considered to be the most toxic and a known carcinogen among all aflatoxins;

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affecting prostate, gastrointestinal and breast cancers (Bbosa, Kitya *et al.* 2013). International Agency for Research on Cancer (IARC) has placed Aflatoxin B1 among the most dangerous carcinogenic agents (Baan and Straif 2021).

Animals that are fed with aflatoxin B1 diet may not excrete it completely and it may remain in their major organs and other tissues of animal and become a hazard to human health. AFB1 exposure by animal sources has already been reported to highlight the danger of such contamination to public health (Wild and Gong 2010, Cardwell 2000, Herzallah, Al-Ameiri *et al.* 2014). Various countries have established and regulated standard limits of aflatoxin in food (Van Egmond and Jonker 2004). In Pakistan, maximum allowed limits for aflatoxins in human food is prescribed as 10µg/kg (Ashiq 2015).

Keeping in view significant health hazards of aflatoxins and to ensure a sound and safe supply of food products, the aim and purpose of current study was to determine the level of aflatoxin in the chicken livers of locally produced broilers in Karachi, Pakistan. The expected results of this study will highlight the danger of such contamination and may cause to prevent accumulation of aflatoxin in the broiler chicken sold in the city.

Material and methods

Samples Collection

120 samples of broiler chicken liver were purchased from 24 chicken slaughtering shops located at different areas of Karachi. The samples were stored at −20ºC until analysis was carried out. The samples were extracted and were investigated in triplicate.

Chemicals and Reagents

Standards of Aflatoxin B1 (analytical grade) were stored at 4ºC prior to use. HPLC grade methanol and acetonitrile (99.9%) were used for analysis. ASC grade glacial acetic acid, potassium chloride, potassium, sodium chloride, dihydrogen phosphate and disodium hydrogen orthophosphate were purchased from Merck. Phosphate-buffered saline (PBS) was prepared according to procedure prescribed earlier (Maniatis 1989). 8 g of NaCl was added to 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 1L ultrapure water and the pH was adjusted to 7.4 with HCl. Double distilled water was used for the preparation of solutions. All other reagents were reagent grade.

Extraction of Aflatoxin

Homogenization of defrosted (25g) sample was carried out. The sample was then blended with 20% aqueous citric acid (2.5ml) of solution and diatomaceous earth (5g). 50ml of dichloromethane were added to the blended mixture and was filtered after 30min shaking. 10ml of Na_2SO_4 was added to the filtered extract and was heated to dryness. 10 ml of acetonitrile: H_2O (75:25, V/V) were added to the dried residue and extracted with 5ml hexane. 5ml of the bottom layer was run off and collected in a container placed under the tap and was heated to dryness. The concentrate was reconstituted with 50ml methanol: water (80:20, V/V). The filtration was carried out by Whatman No. 1 filter paper and the filtrate was diluted to six times with the addition of already prepared phosphate buffered solution (pH=7.4). Immunoaffinity column with a flow rate of 2-3 mL/min was used to elute. Washing of the column was carried out with 30mL distilled water

and then with phosphate buffered solution (pH=7.4), and elution of aflatoxin was carried out with 4mL methanol. The elute was then dried at 40°C under N_2 atmosphere. The dried residue was re-dissolved in 1mL 2:3 vol/vol mixture of methanol and water and derivative with 700µL trifluoroacetic acid: acetic acid: water (20:10:70). It was then filtered through 0.45µm membrane and was stored at -18°C until HPLC analysis (Toxins 2000).

HPLC Analysis

Reverse-phase HPLC (model LC-10ADvp solvent delivery system; auto injection, Shimadzu, Japan) C18 Brownlee reverse phase column (220x4.6mm, particle size 5μm) with C18 guard column (Perkin Elmer) was used with fluorescence detection set at 425 nm emission for aflatoxin B1. The mobile phase waswater:acetonitrile:methanol (66:17:17, v/v/v) with 4M nitric acid and 119mg/L KBr. The oven temperature was maintained to 40°C with a flow rate of1mL/min and injection volume for standard and sample extracts was kept 30μL. Since aflatoxins are possible carcinogen, care has always been practiced to avoid exposure and 10% sodium hypochlorite was used for decontamination.

Statistical Analysis

Standard deviation was estimated by using one way analysis of variance (ANOVA) according to AOAC guidelines. Calibration curves and linear regression curve showed r^2 values above 0.999 for each mycotoxin indicating good linearity.

Detection limit and Validation

HPLC method for the quantitative determination of aflatoxins B_1 has been validated as described earlier (Muscarella, Iammarino *et al.* 2009). The chromatographic separation of aflatoxins was accomplished using a C_{18} column eluted with an isocratic mobile phase consisting of water, methanol and acetonitrile. The sample preparation required a simple extraction of aflatoxins with MeOH/H_2O (80:20, v/v) and a purification step by immunoaffinity column cleanup. The total analysis time, including sample preparation and chromatographic separation, did not exceed 40 min with a run time of 10 min. The procedure for the determination of aflatoxins was extensively validated following Regulation (EC) No. 882/2004 of the European Parliament and of the Council (van Egmond, Schothorst *et al.* 2007).

Results and Discussion

Retention Time

The retention time for Aflatoxins $B_1$was obtained as 5.39minutes.

Detection limit and calibration solutions

Limits of Detection (LOD) are the baseline to measure occurrence of aflatoxin. LOD of Aflatoxins for the chicken liver samples was obtained and was estimated as three times signal-to-noise ratio. The calibration solution of aflatoxinsB1 ranging from 0, 0.025, 0.05, 0.125, 0.25, 0.5, 1.25 ppb were prepare din 1 mL 2:3vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45µm. Seven point calibration curve of peak versus concentration mg/L was constructed for every standard solution. Calibration curve constructed for Aflatoxin B1 is shown as Figure 1.

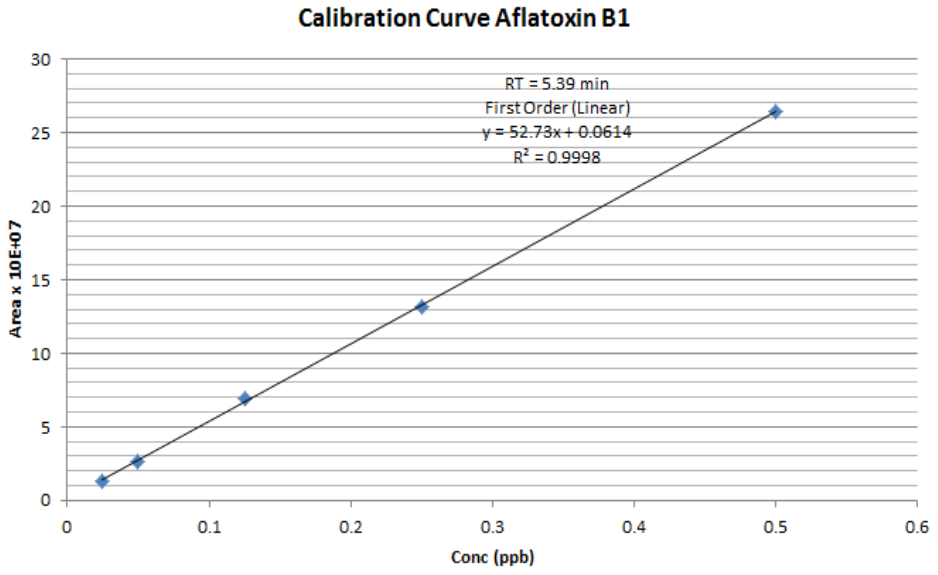

Figure 1: Calibration Curve of aflatoxins B1

Aflatoxin B1 in Chicken Liver Samples

The analytical results of occurrence of aflatoxin in chicken liver samples are summarized in Table 1. A total of 120 chicken liver samples were analyzed for contamination of aflatoxin. Occurrence of aflatoxin was detected for 49 (40.38%) out of 120 of the samples evaluated. Frequency of samples found positive for aflatoxin B1 is shown in figure 2.

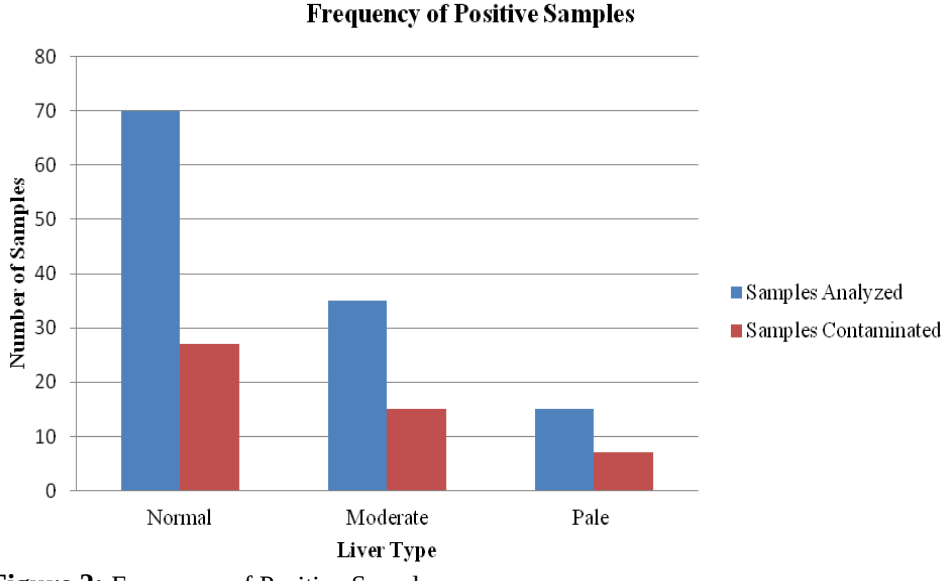

Figure 2: Frequency of Positive Samples

27 (38.57%) out of 70 samples of normal, 15 (42.85%) out of 35 samples of moderate and 7 (46.67%) out of 15 samples of pale colored chicken liver were found positive. Maximum level of aflatoxin $B_1$was detected as 5.02µg/kg for pale colored chicken liver and minimum level 2.38µg/kg in normal chicken liver.

Table 1: Aflatoxin B1 in Chicken Liver Samples

Type of Liver	Samples Analyzed	Samples Contaminated	Positive %	Mean±SD (µg/kg)	Concentration range (µg/kg)
Normal	70	27	38.57	3.54 ± 0.97	2.38–4.68
Moderate	35	15	42.85	3.92 ± 0.88	3.02–4.50
Pale	15	7	46.67	4.55 ± 1.09	3.79–5.02
Total	120	49	40.83	3.80 ± 4.85	2.38–5.02

Discussion

In the current study relatively moderate levels of contamination of AFB1 have been found in chicken liver. It is evident from previous studies that aflatoxin are transferred into chicken liver through chicken feed (Mondlane, Capece *et al.* 2005, Hussain, Khan *et al.* 2010).

The findings of current studies are found to be higher to the results reported for a study carried out for chicken liver samples in which 60 chicken livers samples were analyzed and level of AFB1 was reported to be 2.5µg/kg (El-Desouky, Mohamed *et al.* 2014). Aflatoxin B1 in chicken liver reported earlier, where 39% of the samples were found to contain aflatoxin B1 with a maximum level of 1.73µg/kg (Sineque, Macuamule *et al.* 2017).

The findings of current studies are comparable to a study in which the chicken livers were found to contain 2.98±0.76µg/kg of aflatoxin B1 (Iqbal, Nisar *et al.* 2014). The current study is also comparable to another study, in chicken liver samples were found to contain 3.2µg/kg of aflatoxin B1 (Rodríguez-Amaya and Sabino 2002). AFB1 concentrations in livers samples were found to be 2.41 µg/kg in an investigation (Stamford, Vilar *et al.* 2005).

Although no co-relation of feed and aflatoxin accumulation have been investigated in this study, the levels of AFB1 in chicken liver samples obtained in current study indicate expected mishandling of chicken feed. It is not safe to make deduction on results from a limited sampling in this study. However, the current study pointing out the incidence of presence of aflatoxins B1 in the chicken liver samples purchased from various outlets with slaughtering facilities in Karachi, hence, during the period of study may be regarded as hazardous to human health.

The contamination of aflatoxin has become a serious health threat to humans. Toxic effects due to contamination of aflatoxin such as liver cancer and lowering in immune response in various animals and humans have already been reported earlier (Jiang, Jolly *et al.* 2005, Williams, Phillips *et al.* 2004). AFB1 is known as the most toxic and carcinogenic natural toxicant, which may cause aflatoxicosis and/or induce liver cancer. Aflatoxin B1 also affects with the incidence of gastrointestinal, prostate, and breast cancers; deficiency of protein (Bbosa, Kitya *et al.* 2013).

Conclusion

It can be concluded from the current study that the chicken liver samples obtained from Karachi are contaminated with aflatoxins B1. A total of 120 chicken liver samples were analyzed for contamination of aflatoxin. Occurrence of aflatoxin was detected for 49 (40.38%) out of 120 of the samples evaluated. 27 (38.57%) out of 70 samples of normal, 15 (42.85%) out of 35 samples of

moderate and 7 (46.67%) out of 15 samples of pale colored chicken liver were found positive. Maximum level of aflatoxin $B_1$was detected as 5.02µg/kg for pale colored chicken liver and minimum level 2.38µg/kg in normal chicken liver. The findings of current study (5.02µg/kg maximum and 2.38µg/kg minimum level) are found to be lower than the prescribed standard limits of aflatoxin B1 10µg/kg in human food. The Study provides important evidence of contamination of chicken liver with aflatoxin but at a very limited sampling and in the period of study. The contamination of aflatoxin in chicken liver could be a serious problem for the largest populated city of Karachi, where limited resources are available for the prevention and controlling their levels in the food supply. The authorities should also take steps for strict implication of prescribed standards and to carryout monitoring of aflatoxin contamination in the chicken produced in poultry farms at Karachi, aiming to reduce the toxic effects of such toxins to human health.

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