AFLATOXIN M1 INDUCING GENOTOXICITY AND PATHOLOGICAL LESIONS IN ORGAN MEATS, LIVER OF CATTLE SAMPLED FROM EL-BASATIN ABATTOIR, EGYPT

Islam Elgohary¹; Reham M. Abd-Elsalam²; Nasser Gamal Fadal¹; Mahmoud, A.M. ²*

Address(es):
¹Department of Pathology, Animal Health Research Institute, Dokki, ARC, Giza, 12619, Egypt.
²Department of Pathology, Faculty of veterinary Medicine, Cairo University, Giza, 12211, Egypt.

*Corresponding author: mahnoudaly@cu.edu.eg

ABSTRACT

In this study, two hundred and thirteen liver samples were randomly collected from different ages and both sexes of cattle carcasses in El-Basatin abattoir, Cairo governorate, Egypt. One hundred ninety-seven samples out of the examined specimens were free from any bacterial infections; but showed enlargement and pallor. Thirty-nine specimens representing about 20% of the bacteria-free specimens were examined to detect the Aflatoxins and their metabolites using thin layer chromatography (TLC) and LC MS/MS. All pathological appraisals were recorded and the genotoxicity of the studied mycotoxin was detected using single cell gel electrophoresis (Comet). Our study revealed that there were prominent deleterious effects of Aflatoxin M1 on the hepatic tissue. The detection of aflatoxins’ metabolites in the hepatic tissue is of great importance on the health of human consuming organ meat; and on the other hand, it may affect the farm animals’ performance.

Keywords: Aflatoxins, liver, TLC, LC MS/MS, Comet, Cattle, Histopathology

INTRODUCTION

The liver plays a vital role in mammalian digestion and metabolism; therefore, any fault in the hepatic tissue will affect on the animal health status initiating severe economic losses in farm animal production. Aspergillus fungi, particularly Aspergillus flavus and Aspergillus parasiticus, create aflatoxins (AFs), which are naturally occurring mycotoxins, it grows in stored food commodities (Grassi et al., 2007; Bryden, 2012; Streit et al., 2012). Aflatoxins had hepatotoxic, carcinogenic, teratogenic, mutagenic and immunosuppressive action (Oguz et al., 2003) and also decrease reproduction of animals (van de Walle et al., 2010). In addition to, synergistic action with various pathogenic agents (Mozafari et al. 2017; Imran et al., 2019; Saleemi et al., 2020), aflatoxins decrease feed utilization, poor weight gain, gastrointestinal dysfunction, kidney damage, embryonic death, reduced productivity, anemia, jaundice and death (Pier, 1992). About 17 recorded isolated types of aflatoxins, only 4 of them; B1, B2, G1 and G2; are studied extensively from a toxicological point of view; Aspergillus flavus produce only aflatoxins B1 and B2, whereas Aspergillus parasiticus produce aflatoxins B1, B2, G1 and G2. In humans and animals, aflatoxin M1 is a metabolite of aflatoxin B1. In the milk of cattle given contaminated diets, aflatoxin M2 is a metabolite of aflatoxin B1 (Dhanasekaran, 2011). Swine, chickens, turkeys, and ducklings are more susceptible to aflatoxicosis comparing with sheep and cattle which are more resistant. After ingestion of aflatoxins, they were swiftly absorbed from the gastrointestinal tract then bio-transformed by cytochrome P450 (hepatic mixed-function oxidase system) producing highly reactive epoxides which bound the nucleophilic cell sites forming adducts causing impairment of protein, DNA and RNA synthesis (Benkerroum, 2020). Environmental stress, sex, age, and breed variations can also additionally play a position of their toxicities (Richard, 2008; Parsons and Munkvold, 2010; Rawal et al., 2010). Also, the use of improper stored forage (silage), roughages and cereals may increase the possibility of Aflatoxicosis in ruminants (Binder et al., 2007; Keller et al., 2013; Storm et al., 2014).

The liver is the essential target organ of acute aflatoxicosis in all species. It has been known that acute, subclinical and chronic disease forms have been widely described as clinical aflatoxicosis forms (Benkerroum, 2020). Many outbreaks of acute aflatoxicosis had been reported in bovines (Kaleibar and Helan, 2013; Hernandez-Valdivia et al., 2020) and the most relevant clinical signs and lesions were fatty liver and coagulation disability (Newman et al., 2007). Moreover, exposure to high level of aflatoxin causes alteration in digestion, absorption and metabolism of nutrients (Van de Walle et al., 2010). Chronic aflatoxicosis is similar but less evident (Hernandez-Valdivia et al., 2020). Chronic or subclinical exposure does not cause visible signs, but leads to carcinogenesis, metabolites of aflatoxins can intercalate into DNA (Bennett & Klich, 2003).

In Egypt, Liver is an edible part and could be consumed in different forms of cooking. So, in this study, great attention has been considered to detect the aflatoxin metabolites in the hepatic tissue with special references to pathological and genotoxicity of such toxins on the cattle carcasses.

MATERIAL AND METHODS

Sampling

During the period extended from August 2014 to August 2015, two hundred and thirteen liver samples from different ages and both sexes of cattle carcasses were randomly collected from El-Basatin abattoirs, Cairo governorate, Egypt. After excluding samples contained bacterial and parasitic infection either by isolation and/or detection, the remaining One hundred ninety-seven samples were grossly examined and thirty-nine specimens representing 20% were presented for toxicological examination. Each specimen was divided into three parts, 1st part was collected into plastic bag for detection of aflatoxins by thin layer chromatography and LC MS/MS, 2nd part collected in phosphate buffer saline for single cell gel electrophoresis (comet) and 3rd part was fixed in 10% neutral buffer formalin for histopathological examination.

Thin Layer Chromatography (TLC)

The thin layer chromatography was performed according to (Richard et al., 1993). Representative positive samples for aflatoxins by TLC were examined using liquid chromatography- mass spectrometry (LC MS/MS) to quantify the mycotoxin concentration in the hepatic tissue.

LC MS/MS

The representative positive samples by TLC were examined using LC MS/MS according to (José Diana et al., 2009) where the obtained solution was centrifuged for 10 min at 14000 g in a Ultrafree® MC centrifuge then subjected to LC-MS/MS analysis.

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Single Cell Gel Electrophoresis (COMET)

Comet assay was performed according to (Singh et al., 1988; Rojas and Lopez, 1999). The grading of destruction DNA damage was divided into 5 grades, depending on tail length as a proportion of total length. Grade 0 with no damage, tail length <5%. Grade I with slightly damaged, tail length 5 to 20%. Grade II with moderately damaged, tail length 20 to 40%. Grade III with heavily damaged, tail length 40 to 95%. Grade IV: totally damaged, tail length >95%.

Histopathological examination

Specimens from livers were immediately fixed at 10% neutral buffer formalin, trimmed, washed in water, dehydrated in ascending concentrations of ethanol, cleared in xylene and embedded in paraffin. Thin sections (4-6 µ) were prepared and stained with hematoxylin and eosin. Masson’s trichrome stain was used for staining of fibrous connective tissue (Bancroft, 2008).

Statistical analysis

The data were statistically analyzed using ANOVA (Kotz and Johnson, 1982).

RESULTS AND DISCUSSION

RESULTS

Thin layer chromatography (TLC)

31 samples out of 39 liver samples were positive for detection of aflatoxins in a percentage of 79.48%. The positive results were detected by formation of bands by using TLC. Figure 1 demonstrated the positive results.

Table 2 Comet length, head diameter, DNA % in head, tail length, DNA% in tail

| Examined parameter | Sample 1 AFS 21.23 ng/gm | Sample 2 AFS 11.97 ng/gm | Sample 3 AFS 93.25 ng/gm | Sample 4 AFS 181.73 ng/gm | Sample 5 AFS 183.01 ng/gm | Sample 6 AFS 83.06 ng/gm | Sample 7 AFS 78.2 ng/gm | Sample 8 AFS 105.79 ng/gm | Sample 9 AFS 86.81 ng/gm |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Comet length       | 18.84±0.34               | 19.1±0.3                 | 17.3±0.38                | 17.82±0.32               | 16.62±0.37               | 19±0.38                  | 21.34±0.3               | 14.96±0.38               | 28.73±0.24               |
| Head diameter      | 15.62±0.38               | 17.36±0.37               | 15.88±0.49               | 16.58±0.35               | 17.52±0.36               | 14.88±0.47               | 17.14±0.36               | 14.52±0.4                | 23.56±0.26               |
| DNA % in head      | 95.49±0.4                | 91.38±0.27               | 97.18±0.54               | 95.59±0.38               | 95.46±0.47               | 91.59±0.3                | 94.97±0.36               | 95.15±0.41               | 89.53±0.31               |
| Tail length        | 3.33±0.62                | 2.24±0.4                 | 2.24±0.5                 | 1.88±0.58                | 2.1±0.6                  | 4.1±0.49                 | 4.2±0.42                 | 0.94±0.89                | 5.16±0.39                |
| DNA % in tail      | 4.51±0.4                 | 8.62±0.27                | 2.82±0.54                | 4.41±0.38                | 4.54±0.47                | 8.41±0.3                 | 5.03±0.36                | 4.85±0.41                | 10.47±0.31               |

Legend: A: Aflatoxin’s metabolite concentration. Each value represents mean ± SE; n= 18. Small letters a,b,c,d,e,f,g,h,i in the same rows represents a significant change to capital letters A,B,C,D,E,F,G,H,I respectively by LSD using ANOVA at P≤ 0.05.

The results were categorized according to the tail length as shown in Figure 2. The findings showed that, DNA of all samples were affected by aflatoxin but some of them were with wide variety of destruction as low level of damages (Figures 2A, 2B, 2C, 2E) while others showed high damage (Figures 2D and 2F).

The interpretation of Comet assay depended on tail formation on gel electrophoresis was summarized in Table 2.

Figure 2 DNA damage detected by comet assay. A. Picture showing category 1, cell with low level of damage, head diameter 16.2 µm and tail length 2.52 µm; B. Picture showing category 1, cell with low level of damage, head diameter 14.76 µm and tail length 0.72 µm; C. showing category 1, cell with low level of damage, head diameter 25.56 µm and tail length 3.6 µm; D. Picture showing category3, cell with high damage level, head diameter 16.2 µm and tail length 15.48 µm; E. Picture showing category1, cell with low level of damage, head diameter 31.68 µm and tail length 6.48µm; F. Picture showing category 3, cell with high damage level, head diameter 27.72 µm and tail length 18.72µm. Regarding tail length in liver cells of cattle is significantly the highest in liver cells have concentration of 83.06 ng/gm Aflatoxin M1, then in liver cells have concentration of 86.81 ng/gm Aflatoxin M1, then in liver cells have concentration of 78.2 ng/gm Aflatoxin M1, then in liver cells have concentration of 105.79 ng/gm Aflatoxin M1.
Pathological findings

Gross examination

The examined liver samples of some cases were apparently normal but others were pale and enlarged with sub capsular hemorrhage in some areas. The hepatic tissues of such cases were friable and appeared with focal areas of necrosis. Thickening of hepatic capsule was not common.

Histopathological findings

The positive aflatoxins samples showed that, some hepatocytes were vacuolated (Figure 3A) while others showed severe lesions characterized by necrosis. Necrobiosis changes in the hepatocytes were common finding in the area of necrosis where marked pyknotic and karyolitic nuclei were noticed (Figure 3B). The hepatic capsule in some examined cases showed marked thickening with fibrosis indicating Glisson’s cirrhosis (Figure 3C). In other cases, congestion in hepatic sinusoids with activation of von Kupffer cells was prominent (Figure 3D).

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DISCUSSION

In our study, 31 samples out of 39 were positive for aflatoxin (79.48%) by using TLC. The concentration of aflatoxin M1 in representative samples using MS/MS indicates different concentration values. The gross examination of these samples showed liver enlargement, pale color and in some cases presence of subcapsular hemorrhages. Such findings were agreed with (Percyra et al., 2008) who showed that the livers of animals affected by aflatoxicosis revealed size enlargement, pale coloration, rounded borders, and expanded friability. The histopathological examination revealed that hepatocytes showed multiple vacuoles while other hepatocytes showed necrobiosis changes as pyknosis and karyolysis which were in agreement with previous studies of (Colakoglu and Donmez, 2012) who showed that vacuolar degeneration and pyknotic nuclei in the hepatocytes in some lobules were common in aflatoxicosis. The same results were achieved by (Devendra et al., 2011) who showed the effect of aflatoxin on the hepatocytes was appeared as severe hydrophilic and vacuolar degeneration in the most of hepatocytes with disseminated necrotic cells. In the present study, Portal fibrosis and endophlebitis were the common findings which characterized by swelling of endothelial cells and marked thickening in hepatic vein wall that lead to obstruction of hepatic vein in some cases. In addition to that, the bile ducts were hyperplastic with newly formed bile ductules. The same results were recorded by (Colvin et al., 1984) who discussed the Aflatoxicosis in feeder cattle and showed that presence of portal fibrosis with biliary proliferation and venous obstruction as the disease progresses. This results were also in agreement with previous studies of (Rajendran et al., 1992) who made Clinicopathology of aflatoxin toxicity in cattle and showed proliferation of bile duct epithelium, obliteration endophlebitis of centrilobular and hepatic veins (characteristic to bovines) and diffuse fibrosis leading to variation in morphology of hepatocytes. The positive aflatoxin samples appear as bands by using ultraviolet lamb which in agreement with (Grossou et al., 2004) who proved that TLC is used to identify and quantify aflatoxins at very low levels and confirmed by (Truckssess, 2000) who determined the aflatoxins in chinchilla feed samples. The concentration and type of aflatoxins are determined in liver specimens by LCMSMS where high concentration of aflatoxin M1 (AFM1) in these liver sample (Table 1). The mycotoxin metabolite concentration exceeding the maximum level of AFM proposed by the WHO (0.500 µg AFM1/kg), European Regulation (EC) declares a limit of 0.050 µg AFM1/kg milk (Commission Regulation (EC) 2006). Comet results in our study showed destruction on DNA of hepatocytes in the form of head and tail due to Aflatoxins can induce mutagenesis by alkylation of nuclear DNA, leading to carcinogenesis and teratogenesis which is go parallel with the findings of (Hussain et al., 2007) who showed that the carcinogenic, and teratogenic effect occurred due to The reaction with DNA occurs with guanine in the codon 249 of tumor suppressor gene p53 where the G to T trans version occurs. This result was also in agreement with (Wong and Hisieh, 1976) who proved that the limited ability to metabolize AFM1 into the DNA-reactive epoxide may thus account for its ability to cause DNA damage and pre-neoplastic lesions as compared to AFB1. From statistical point of view, our study revealed that, some of the examined samples showed marked increase of the DNA fragmentation with the increased mycotoxin concentration as in sample No. 5 where the mycotoxin concentration was 183.01 ng/gm the tail length in comet assay was 2.1± 0.6, in sample No. 8 where the concentration was 105.78 ng/gm and tail length was 0.94± 0.89 on the other hand in other specimens although the concentration of mycotoxins were lower as in sample No. 2,1,7,4 and 9 where the mycotoxin concentration was 11.79, 21.23,78,2.81,73and 86.81ng/gm respectively , the tail length in comet assay was ( 2.24± 0.7, 3.33± 0.62, 4.2 ± 0.42, 1.88 ± 0.58 and 5.16± 0.39). In this case, the role of stress and breed differences may play role in its toxicities which confirmed by (Richard, 2008; Parsons and Munkvold, 2010; Rawal et al., 2010).
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4
