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Original Article

The Expression of MMP1 and MMP7 in Mice Liver after Exposure to Aflatoxin B1 Using Immunohistochemistry Technique

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Abstract

Mycotoxin is a class of poisonous secondary metabolites generated by filamentous fungi and found in agricultural commodities worldwide. Therefore, the current study aimed to investigate how aflatoxin B1 affected hepatic cellular architecture and Matrix metalloproteinase expression in particular (MMP1 and MMP7) in the livers of experimental mice (IHC). A total of sixteen mice (four groups) were studied after being given pure aflatoxin B1 (9mg/kg B.W., 6mg/kg B.W., and 3mg/kg B.W.) (produced from Aspergillus flavus) or a control group (not treated). MMP1 and MMP7 expressions were also measured using the MMP1 and MMP7 expression assays (IHC). The degree of liver damage is related to the AFB1 concentration and the duration of exposure. IHC reveals a considerable rise in MMP1 and MMP7 expression in the livers of mice given a maximum concentration of 90% ((9 mg/B.W.) pure AFB1), which approached the toxin's effect toxic dosage. MMP1 and MMP7 expression were also increased by AFB1 at dosages of 60 and 30% (6mg/BW and 3mg/B.W., respectively), although not to the same extent as 90%. MMP1 was significantly more expressed than MMP7 compared to control, and AFB1 at 90, 60, and 30% concentrations caused changes in hepatic cellular architecture, organization, and liver tissue damage and dramatically increased MMP1 and MMP7 production in hepatic tissue following treatment. Increased levels of pure aflatoxin B1 will harm liver tissue and MMP1 and MMP7 expression. MMP1 was more substantially expressed than MMP7.

Keywords: Matrix metalloproteinases MMP1 and MMP7, Aflatoxin B1, Immunohistochemistry technique

1. Introduction

Mycotoxin is a class of poisonous secondary metabolites generated by filamentous fungi and found in agricultural commodities worldwide. Mycotoxin is harmful to both animal and human health. Aflatoxin has been known as the most deadly mycotoxin; its ubiquitous and persistent presence in food and feed commodities poses a significant threat to animal and human health. Aflatoxin poisoning can cause significant health problems, such as carcinogenesis, mutagenesis growth retardation and immunological suppression (1, 2). Aflatoxin B1 is classified as a class 1 carcinogen by the WHO (3). This toxin's toxicity varies depending on the organ that has been injured, notably the liver. Aflatoxins have previously been connected to illnesses such as aflatoxicosis, other health difficulties, and human health problems. Similarly, the lethal toxicity of Aflatoxin B1 in animals varies, with resistant (Monkey, Chicken, Mouse) and very vulnerable species (Sheep, Rat, Dog) (4, 5).

Matrix Metalloproteinases MMPs are members of the metzincin family, a group of 24 Zn^{2+}-dependent, Ca^{2+}-containing endoproteases found in mammals (23 in humans). MMPs are produced as pro-MMPs

(zymogens) that are activated by other free radicals or enzymes via the cysteine switch mechanism (6). Metalloproteinases have names that begin with MMP-1 and conclude with MMP-28. MMPs are divided into various categories based on the substrate they attack and their chemical formula and structure: MMPs of the membrane type, matrilysins, stromelysins, gelatinases, collagenases, and unidentified MMPs (7). By encouraging the breakdown of particular ECM proteins, MMPs alter the structure of the surrounding tissue and cells. Different MMPs are more or less effective at breaking down different proteins. MMPs target a variety of substrates, including MMP-13, MMP-11, MMP-3, MMP-2, MMP-1, vitronectin, tenascin, laminin, fibronectin, entactin, aggrecan, gelatin, collagen, that along with the MMPs are expressed in normal livers, whereas the others can be involved in the processes of pathology, for instance, chronic or acute liver damage (8). Acute and chronic liver injuries are typical hepatic injury categories, and MMPs have been linked to various acute and chronic liver disorders. Acute liver injury can be caused by a number of things, including medications, bile acids, toxins, ischemia/reperfusion events, and infections. Acute oxidative stress causes cellular damage that generates an immune response that, if left uncontrolled, can lead to significant cell death (9).

MMP-1 is a metalloproteinase type called collagenase-1 because it speeds up the breakdown of interstitial collagens or fibroblast collagenase. MMP-1 is controlled by TIMP-1, which is a tissue inhibitor of metalloproteinases.MMP-1 performed a role in the repair of liver fibrosis in animals (10). MMP-1 is known to increase tissue fibrosis in patients with NASH (nonalcoholic steatohepatitis), indicating that it may function in liver repair and regeneration. MMP-1 and TIMP-1 overexpression is associated with an increase in the migratory capability of HCC (Hepatocellular carcinoma) cells, most likely as a result of ECM degradation during the epithelial-mesenchymal transition (EMT) (11). MMP-7 has also been demonstrated to trigger MMP-1 (12).

MMP-7, also known as elastin, laminin, and fibronectin, is a protein that cleaves collagen, matrilysin, osteopontin, proteoglycans, and entactin, as well as pro-MMP-9 and pro-MMP-2 and other extracellular matrix proteins (6). MMP 7 is primarily connected to tissue remodelling in biliary atresia-related liver fibrosis.MMP7 expression has been raised in several human primary malignancies, including lung, breast, ovarian, and prostate cancer. Both benign and malignant colorectal tumours have been reported to up-regulate MMP7 (10).

Therefore, the current study aimed to investigate how aflatoxin B1 affected hepatic cellular architecture and Matrix metalloproteinase expression in particular (MMP1 and MMP7) in the livers of experimental mice (IHC).

2. Materials and Methods

2.1. Aflatoxin B1

Aflatoxin B1 synthesis, extraction, and purification were done according to the method previously described byAl-Mudallal, Almashta (13). The toxin was made from a pure culture of *Aspergillusflavus* recovered from patients with aspergillosis.

2.2. Determination of Aflatoxin B1 Toxic Effects

According to Park and Troxell (14), a harmful impact dosage of 9mg/kg B.W. of aflatoxins B1 pure extract was determined based on early experiment data and reflected a 90% aflatoxin concentration. From this concentration, further concentrations (30 and 60%) of pure aflatoxin B1 were used, equating to 3mg/kg B.W. and 6mg/kg B.W., respectively.

2.3. Animals

Sixteen (male) Swiss albino mice aged 10 to 12 weeks were randomly divided into 4 equal groups (n=4). Before being treated, the mice were given a two-week acclimation period. They were in plastic cages with complex wood chips for bedding in a climate-controlled animal home with a 4/10 hour light/dark cycle. The animals were given water and a balanced feed in suitable quantities. They were housed at the

Biotechnology Research Center/Al Nahrain University animal house in Baghdad, Iraq.

2.4. Experimental Design

Sixteen mice were fed orally and divided into four groups to assess the histological and immunohistochemical effects of aflatoxin B1 on the mice's liver.

Group 1: Control group, which received no treatment.

Group 2: Animals were fed a pure extract of aflatoxins B1 (9mg/kg B.W.) twice a week for 2 weeks (approximately 90% of the concentration).

Group 3: Animals were gavaged with a single dose of purified aflatoxins B1 extract for 10 days (6mg/kg B.W.) (representing 60% of concentration).

Group 4: Animals were gavaged once daily with a purified extract of aflatoxins B1 for 10 days (3mg/kg B.W.) (Representing 30% of the concentration).

2.5. Histopathological Study

A histopathological study was done, as stated by Bancroft and Gamble (15), and each mouse was dissected after having its blood collected from anaesthetized animals. After removal, the livers were placed on a petri dish containing a physiological solution with pH adjusted to 7. This solution was made in accordance with Benson (16).

Mayer's albumin was used to attach tissue slices to slides, which were dried in a 37oC oven for 1–2 hours. Before mounting the sections on slides, the xylene-dewaxed tissue was washed three times with 10% alcohol and then with 70 and 95% alcohol. The slides were stained with Eosin and Hematoxylin for about 12 seconds after being rinsed in water for 5 minutes, washed in water for 3 minutes, and then dipped in acid alcohol at 1% concentration.The slides were dried after drying and then washed with (70, 80 and 95%) alcohol for only a few seconds. Before being coated with Distyrene-Plasticizer-Xylene DPX, the slides were soaked in Xylene for 15–30 minutes. The light microscope was used to do the histological examination.

2.6. Estimation of MMP1 and MMP7 by Immunohistochemical Technique

MMP1 and MMP7 expressions in the affected liver were examined using immunohistochemistry (IHC). Slices of the afflicted liver were deparaffinized for one hour (seventy minutes) in a hot air incubator at 80°C using adhesion microscope positively charged slides and then rehydrated in graded alcohols.Backing slides were submerged in the following solutions in the order given for the times specified at room temperature: 30 minutes of Xylene, 30 minutes of fresh Xylene, 5 minutes of absolute ethanol, 90% ethanol, 70% ethanol, 50% ethanol, and 5 minutes of distilled water. Immunohistochemistry was performed on these sections using polyclonal anti-MMP1 and anti-MMP7 antibodies. After cooling the slides for 20 minutes at room temperature to prevent the contents from seeping out during the IHC staining run, a pap pen liquid blocker was used to define the borders of the sections. The slides were rapidly shifted to the strainer racks to avoid drying the samples. To avoid nonspecific background staining, protein block 20L was added and incubated at room temperature for just 10 minutes before being rinsed twice with buffer solution.

About 40µL of primary antibodies (anti-MMP1) (cat. no. ab52631; Abcam, Cambridge, UK) and (anti-MMP7) antibodies (cat. no. ab5706; Abcam, Cambridge, UK) were applied to tissue slices separately and incubated at 37°C for half an hour, in a humidified chamber. Before being stored in the refrigerator for 24 hours, the slides were gently drained and blotted. After one day (exactly 24 hours), rinse the slides and wash them for 5 minutes in a buffer bath, drained and gently blotted, and 20µL of the secondary antibody (the complement) was applied to the sections, incubated for 10 minutes at 37°C, rinsed and placed in washing buffer bath as before, excess buffer drained and gently blotted, then 20µL of HRP(horseradish peroxidase)conjugate was added to each section of tissue and incubated for 15 minutes at 37°C in a

humidified room 3,3'-diaminobenzidine tetrachloride (DAB).

DAB Substrate was mixed with Chromogen (50 drops to 1 drop) and applied to tissue for 1-10 minutes before being rinsed four times in buffer. After being immersed in a bath of Mayer's Hematoxylin for only one minute and then washed 3 times with distilled water (each time one minute), the slides were drained and gently blotted; then dried by placing the prepared slides in the following solutions for 5 minutes: 50% ethanol, 70% ethanol, 90% ethanol, absolute ethanol, Xylene, and fresh Xylene each of them for 5 minutes. Before drying overnight, a drop of dibutyl phthalate in Xylene (DPX) was given to the wet xylene portions, which were gently covered with slips to eliminate the surplus and air bubbles.

2.7. Immunostaining Evaluation for MMP1 and MMP7 Expressions

The expression of MMP1 and MMP7 was measured in each control and treated AFB1 segment by counting the number of cells with positive MMP1 and MMP7 that had a brown (DAB) cytoplasmic staining. Immunostaining for MMP1 and MMP7 expressions was quantified using a scoring system that measured the extent and intensity of staining in three fields of hotspot areas at a power magnification of 40X, scored from 0-100 per cent, and divided into weak, moderate, and strong groups, as shown in tables 1 and 2 (17-19).

Table 1. Scoring system for MMP1 immunostaining expression

MMP1	Score	Intensity	Stained cells (%)
Negative	-	No staining	0
Positive	+	Weak	<70
	++	Moderate	>70
	+++	Strong	>80

Table 2. Scoring system for MMP7 immunostaining expression

MMP7	Score	Intensity	Stained cells (%)
Negative	-	No staining	0
Positive	+	Weak	<40
	++	Moderate	≥40
	+++	Strong	>50

2.8. Statistical Analysis

The available statistical tool, SPSS-27, was used to analyze the data (Statistical Packages for Social Sciences- version 27). Simple measurements of mean and standard deviation were used to present the data. Students'-test for the difference between two independent means was used to determine the significance of the difference between various means (quantitative data). Statistical significance was evaluated when the *P*-value was equal to /or less than 0.05 (20).

3. Results

3.1. Histological Study

Histological examination of the control mice's liver section shows normal liver histology in the control group (Figure 1A). While histopathological examination of the liver from mice treated with 30% (3mg/kg B.W.) of AFB1 single dose daily (for ten days) shows sinusoidal congestion (black arrow) with hydropic degeneration of scattered hepatocytes (red arrow) (Figure 1B). The third section from mice treated with 60% (6mg/kg B.W.t) AFB1 single dose daily (for ten days) shows hyperemia (black arrow), sinusoidal contraction, and a few hepatocytes with hydropic degeneration (yellow arrow), and pyknotic nuclei (blue arrowhead) (Figure 1C). The final histopathological section of mice liver treated with 90% AFB1 single dose daily (for ten days) shows necrosis (red arrow) and melanocytosis (black arrow), with few PMNs.

3.2. Estimation of MMP1 and MMP7 Using Immunohistochemical Technique

Immunostaining was absent in the control slide but present in the AFB1-treated slides, indicating immunohistochemical signal specificity. Using the Immunohistochemistry (IHC) technique, the investigated limitation was accounted for and regarded as definite data. Therefore it was supplied as a count and percentage. The significance level was set at 0.05. Positive MMP1 and MMP7 Immunostainingwas identified in the cytoplasm of the cells as brown staining (DAB generate brown staining and is stained

with hematoxylin). Results shown in figures 1 and 2 and table 3 demonstrated that positive IHC expressions for both markers were found in all treated AFB1 liver tissue sections compared to control (no staining was detected). The number of staining cells increased with the increase in the concentrations of AFB1. For MMP1 at concentrations (30, 60 and 90%) AFB1, the recorded expressions (66.12±6.18, 84.00±1.00 and 90.00±1.00) are shown in figures 1B, 1C and 1D) respectively, and table 1 compared with the control (no staining) figure 1A. For MMP7 at concentrations (30, 60 and 90%) of AFB1, the expressions were recorded to be 36.20±3.30, 40.03±3.95 and 67.33±3.51 as shown in figures 2B, 2C and 2D respectively, and table 3 as compared with the control (no staining) (Figure 2A). The lowest expressions for these markers were detected in 30% AFB1 concentration, while the highest expressions were detected in 90% concentration. In table 3, the *P*-values that compared the AFB1 concentrations (30×60, 60×90 and 30x90) with the expression of MMP1 show a significant correlation (0.008*,0.002* and 0.003*), respectively, while the significance of MMP7 expression was recorded only in 60×90 and 30×90 P values (0.001* and 0.0001*) respectively (Figure 3). By making a comparison between these two markers, MMP1 was more significantly expressed over MMP7, *P*-values (0.002*, 0.0001*, and 0.0001*) at 30, 60 and 90% concentrations of AFB1, respectively (Table 3 and Figure 4). The highest MMP1 expression (90.00) was detected at concentration 90% AFB1, while the highest MMP7 expression (67.33) was detected in 90% AFB1. AFB1 at a concentration of 90% represented the effective dose due to the highest expression of these markers in mice liver tissue.

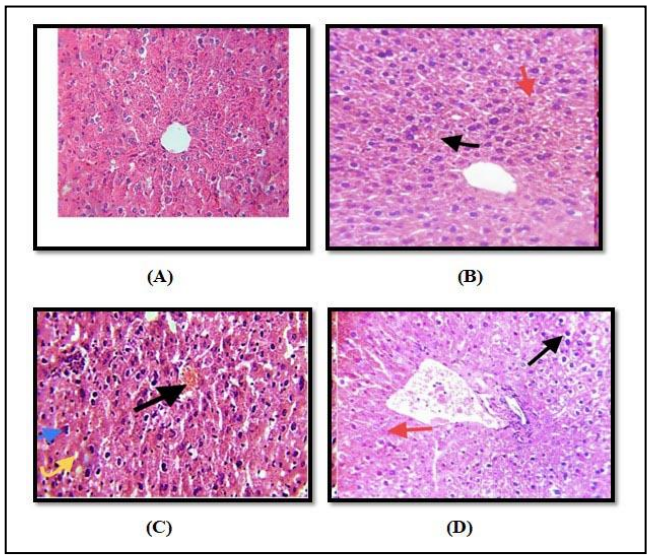

Figure 1. Albino mouse liver tissue sections, (**A**) Normal histology of the liver in the control group without any treatment (H&E ×200), (**B**) Liver histology from an animal treated with 30% AFB1 single dose daily (for ten days), sinusoidal congestion (black arrow) with hydropic degeneration of scattered hepatocytes (red arrow) (H&E ×200), (**C**) Liver histology from an animal treated with 60% AFB1 single dose daily (for ten days), shows hyperemia (black arrow), sinusoidal contraction, and a few hepatocytes with hydropic degeneration (yellow arrow), and pyknotic nuclei (blue arrowhead)(H&E ×200), (**D**) Liver histology from an animal treated with 90% AFB1 single dose daily (for ten days), shows necrosis (red arrow) and melanocytosis (black arrow), with few PMNs. (H&E ×200).

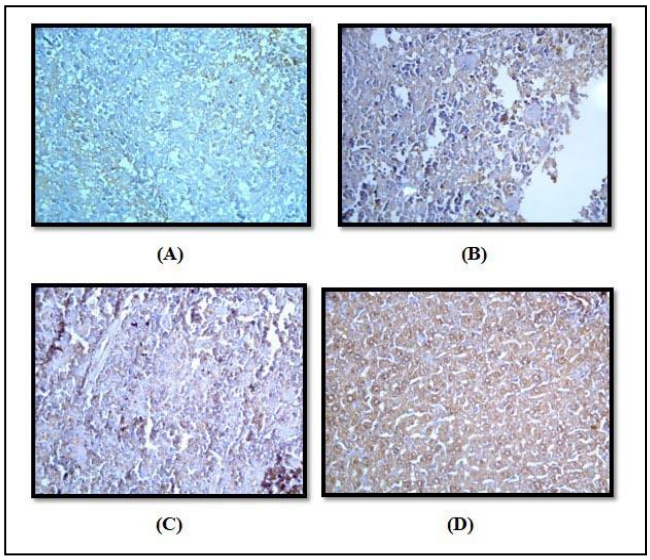

Figure 2. The expression of MMP1 in mice liver sections after being treated with different concentrations of AFB1 (**A**) without any treatment (control)(score -), (**B**) Mouse treated with 30% AFB1 single dose daily (for ten days) (score +), (**C**) Mouse treated with 60% AFB1 single dose daily (for ten days) (score ++), (**D**) Mouse treated with the effected toxic dose 90% AFB1 two times in a week (for two weeks) (score +++), DAB staining (brown), counterstained by hematoxylin (40×).

Table 3. Statically expression of MMP1 and MMP7 in mice liver after exposure to different concentrations of AFB1 (30%, 60% and 90%)

	Aflatoxin concentration			***P*-value comparing**		
	30% Aflatoxin	**60% Aflatoxin**	**90% Aflatoxin**	**30×60**	**60×90**	**30×90**
Score group	+	++	+++			
Mmp1	66.12±6.18	84.00±1.00	90.00±1.00	0.008*	0.002*	0.003*
Mmp7	36.20±3.30	40.03±3.95	67.33±3.51	0.267	0.001*	0.0001*
Control	-	-	-			
P-value	0.002*	0.0001*	0.0001*			

*Significant difference between two independent means using Students t-test at 0.05 level

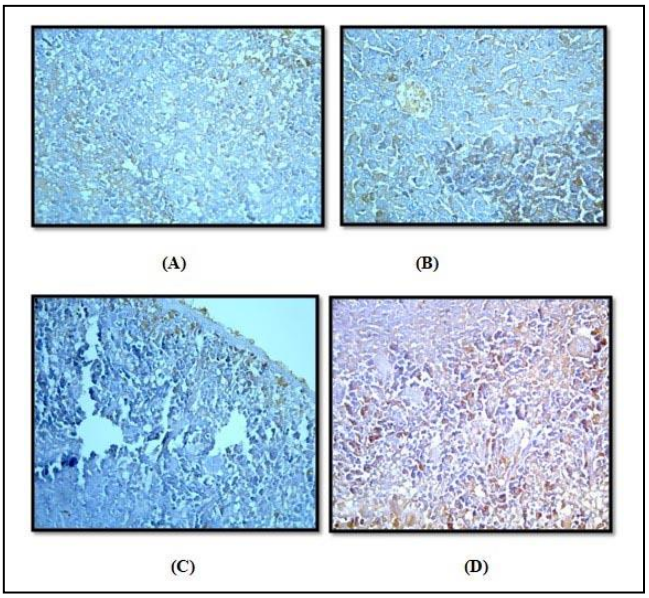

Figure 3. The MMP7 expression in mice liver sections, (**A**) without any treatment (control) (score -), (**B**) Mouse treated with 30% AFB1 single dose daily (for ten days) (score +), (**C**) Mouse treated with 60% AFB1 single dose daily (for ten days) (score ++), (**D**) Mouse treated with the effected toxic dose 90% AFB1 two times in a week (for two weeks) (score +++). DAB staining (brown), counter stained by hematoxylin (40×)

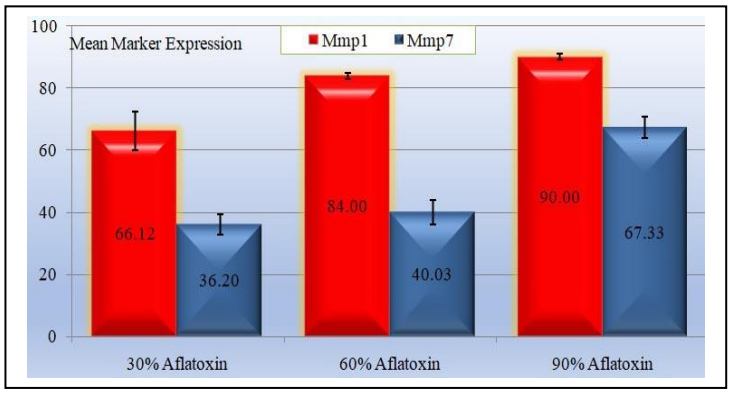

Figure 4. Statically comparison between MMP1 and MMP7 expressions in correlation with different concentrations of AFB1 (30%, 60% and 90%)

4. Discussion

According to the findings of this study, anomalies in hepatic cellular architecture, organization, and liver tissue damage were discovered by histopathological analysis. The amount of AFB1 in the system and the length of time it was exposed were linked to the severity of liver injury (13). There was a significant link between aflatoxins and diseases such as aflatoxicosis, livestock and other health issues in humans worldwide. Animals of all species are vulnerable to aflatoxicosis, and the sensitivity of the animals diverges widely upon the dose, exposure time, species, sex, age, and diet. AFM, AFB2, and AFB1 were identified in the gall bladder, liver, heart, muscle, kidney, and spleen of rising pigs when protein-free and protein diet portions were fed independently (21). C57BL/6 mice were given on the first-day oral dosage of AFB1 (663, 442, or 44µg AFB1 each 1kg of body weight). On the fifth day, liver histology was assessed. The livers of treated mice showed necrosis, inflammatory infiltration, and nuclear vacuolation. Mainly, lesions in animals were treated with three concentrations of AFB1; 663, 442, and 44µg to each 1kg of body animal weight; however, the 663µg of AFB1 shows an additional apparent severity of necrosis, nuclear vacuolation, and melanocytosis, than442, or 44µg AFB1 (22).

The liver is the key organ engaged in AFB1 toxicity and biotransformation (23), regulating the metabolism of amino acids, carbohydrates, and lipids, as well as detoxification and immune protection. The negative consequence induced by AFB1 biotransformation is oxidative stress (24). According to *in vitro* tests, the exposure AFB1 exposure can produce oxidative damage in the primary broiler hepatocytes and human hepatocyte L02 cells (25). AFB1 disrupted the equilibrium between oxidant and antioxidant in the livers of chicken, mice, rats and Nile tilapia (Oreochromisniloticus), causing oxidative biomolecule damage (26, 27).

As a result, oxidative stress was recognized as an essential pathogenic factor in AFB1-induced hepatotoxicity. In vivo and in vitro experiments demonstrated that AFB1 can improve the permeability of the mitochondrial membrane, reducing MMP, and specifically disrupt the cycle of TCA (tricarboxylic acid) and the activities of OXPHOS (oxidative phosphorylation), which might explain hepatocyte damage and liver tissue destruction (28).

Mitochondria are responsible for cellular bio-oxidation, which provides 95% of the ATP required for numerous liver activities. Mitochondrial dysfunction is linked to a number of acute and chronic liver diseases, together with toxicant-induced liver damage (29). Xu, Li (28) demonstrated that AFB1's liver toxicity was linked to reduced mitochondrial biogenesis, aggravating hepatic mitochondrial abnormalities in mice. They concluded that AFB1 exposure lowered liver total and relative weights and caused severe pathological damage to the liver. Immunohistochemical (IHC) elevation for the expression of some matrix metalloproteinases markers that play a role in regenerating the potential of the liver, as confirmed through the capability to control and adjust the mass growth after hepatectomy and recovery from toxic, ischemic, or acute liver infection, which was added to the histopathological examinations of mice livers. However, there is little evidence of a link between AFB1 and these markers in Iraq.

In this research work, immunohistochemistry procedures and techniques were used as the best investigation tool to detect and determine the effects of AFB1. So evaluation of the expressions of MMP1 and MMP7 in the liver was determined. The result of this study indicated that positive IHC expressions for both markers were found in all treated AFB1 liver tissue sections as compared with control (no staining was detected). The expressions of these two markers were significantly raised with the increases in the AFB1 concentrations. The highest MMP1 expression (90.00) was detected at a concentration of 90% AFB1, while the highest MMP7 expression (67.33) was detected at 90% AFB1.AFB1 at a concentration of 90%

represented the effective dose due to the highest expression of these markers in mice liver tissue and the adverse effect of this mycotoxin on hepatocytes, such as necrosis and megalocytosis. By making a statistical comparison between these two markers, MMP1 was more significantly expressed than MMP7.

Hepatocytes, around 80% of the liver's weight and 70% of all liver cells perform most of the liver's metabolic processes. Hepatocytes, endothelial cells, and other liver cells are all especially vulnerable to various insults and play a role in various clinically characterized liver injury syndromes (30).

Matrix metalloproteinases (MMPs) are endopeptidases considered zinc-dependent, which play a role in rebuilding the ECM (extracellular matrix) in pathological and normal conditions. In addition, MMPs can play a role in liver regeneration, as well as a variety of liver illnesses such as HCC, hepatitis, fatty liver, hepatic inflammation, fibrosis and liver cirrhosis (19). Matrix proteins, such as gelatin, fibronectin, collagen, elastin, and non-matrix substrates, such as growth factors, adhesion molecules and chemokines, are processed by MMPs. MMPs are found to play a non-canonical function in various bioactivities, like; wound repair, cirrhosis, angiogenesis, and cancer, in addition to ECM destruction (6).

The functions of MMP7 and MMP1 have been examined after injury and shown to facilitate re-epithelialization, aiding wound healing by breaking down distinct components of ECM (31). MMP1 has also been linked to regulating smooth muscle cell de-differentiation via the protease-activated receptor-1.MMP-1 might contribute to liver repair and regeneration, whereas MMP7 is mainly related to tissue rebuilding and remodelling during biliary atresia associated with the liver's fibrosis (6). These facts explain the increase in the expression of MMP1 in comparison with the MMP7.

This study concluded that AFB1 at 90, 60 and 30% concentrations changed the hepatic cellular architecture, organization and liver tissue damage and significantly elevated the expression of MMP1 and MMP7 in hepatic tissue after treatment compared with the control. The liver tissue damage and expression of MMP1 and MMP7 increased with the concentration increases of purified aflatoxin B1. 90% concentrations of AFB1represents the optimum toxic dose that affects hepatocytes and affects the expression of these Matrix metalloproteinases in mice liver. MMP1 was more significantly expressed over MMP7, according to *P* values<0.05 level comparison. Further studies are needed by increasing the duration of exposure of animal models to AFB1 and determining the histopathological changes and IHC expression of other types of matrix metalloproteinases.

Further studies are needed by increasing the duration of exposure of animal models to AFB1 and determining the histopathological changes and IHC expression of other types of matrix metalloproteinases.

Authors' Contribution

N. M. is responsible for conceptualization, methodology, data curation, formal analysis, original draft, validation, writing review and editing, and finalizing the manuscript.

Ethics

The Ethical Committee of Al-Iraqi University's College of Medicine in Baghdad, Iraq (no. K.T. sh/11) authorized the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Liew W-P-P, Mohd-Redzwan S. Mycotoxin: Its impact on gut health and microbiota. Front Cell Infect. 2018;8:60.
2. Omotayo OP, Omotayo AO, Mwanza M, Babalola OO. Prevalence of mycotoxins and their consequences on human health. Toxicol Res. 2019;35(1):1-7.
3. WHO. Hazardous chemicals in human and environmental health. Geneva, Switzerland: World Health Organization. 2002.
4. Dai Y, Huang K, Zhang B, Zhu L, Xu W. Aflatoxin B1-induced epigenetic alterations: An overview. Food Chem Toxicol. 2017;109:683-9.
5. Muhammad I, Sun X, Wang H, Li W, Wang X, Cheng P, et al. Curcumin successfully inhibited the computationally identified CYP2A6 enzyme-mediated bioactivation of aflatoxin B1 in arbor acres broiler. Front Pharmacol. 2017;8:143.
6. Naim A, Pan Q, Baig MS. Matrix metalloproteinases (MMPs) in liver diseases. J Clin Exp Hepatol. 2017;7(4):367-72.
7. Scheau C, Badarau IA, Costache R, Caruntu C, Mihai GL, Didilescu AC, et al. The role of matrix metalloproteinases in the epithelial-mesenchymal transition of hepatocellular carcinoma. Anal Cell Pathol. 2019;2019.
8. Yamahana H, Terashima M, Takatsuka R, Asada C, Suzuki T, Uto Y, et al. TGF-β1 facilitates MT1-MMP-mediated proMMP-9 activation and invasion in oral squamous cell carcinoma cells. Biochem Biophys Rep. 2021;27:101072.
9. Niland S, Riscanevo AX, Eble JA. Matrix metalloproteinases shape the tumor microenvironment in cancer progression. Int J Mol Sci. 2021;23(1):146.
10. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuna JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. Int J Mol Sci. 2020;21(24):9739.
11. Lai Y-L, Gong C-L, Fu C-K, Yueh T-C, Tsai C-W, Chang W-S, et al. The contribution of matrix metalloproteinase-1 genotypes to hepatocellular carcinoma susceptibility in Taiwan. Cancer Genom Proteom. 2017;14(2):119-25.
12. Djuric T, Zivkovic M. Overview of MMP biology and gene associations in human diseases. Role Matrix Met Hum Body Pathol. 2017;1(3).
13. Al-Mudallal NH, Almashta HIA, Rashid SA, Nakeeb A. Study the Expression of Tumor Growth Factor β (TGF β) in Mice Organs after Exposure to Aflatoxin B1 using Immunohistochemistry Technique. Extraction. 2009;11:12.
14. Park D, Troxell T. Mycotoxins and food safety. Adv Exp Med Biol. 2002;504:173-9.
15. Bancroft JD, Gamble M. Theory and practice of histological techniques: Elsevier health sciences; 2008.
16. Benson. Microbial Applications Laboratory Manual in General Microbiology: The McGraw-Hill Companies; 2001.
17. Wang QM, Lv L, Tang Y, Zhang L, Wang LF. MMP‐1 is overexpressed in triple‐negative breast cancer tissues and the knockdown of MMP‐1 expression inhibits tumor cell malignant behaviors in vitro. Oncol Lett. 2019;17(2):1732-40.
18. Yuan S, Lin L-s, Gan R-H, Huang L, Wu X-t, Zhao Y, et al. Elevated matrix metalloproteinase 7 expression promotes the proliferation, motility and metastasis of tongue squamous cell carcinoma. BMC Cancer. 2020;20(1):1-12.
19. Ito K, Kitajima Y, Kai K, Matsufuji S, Yamada K, Egawa N, et al. Matrix metalloproteinase‐1 expression is regulated by HIF‐1‐dependent and epigenetic mechanisms and serves a tumor‐suppressive role in gastric cancer progression. Int J Cancer. 2021;59(6):1-15.
20. Celentano DD, Platz E, Mehta SH. The centennial of the Department of Epidemiology at Johns Hopkins Bloomberg School of Public Health: a century of epidemiologic discovery and education. Am J Epidemiol. 2019;188(12):2043-8.
21. Marchese S, Polo A, Ariano A, Velotto S, Costantini S, Severino L. Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. Toxins. 2018;10(6):214.
22. Ishikawa AT, Hirooka EY, Alvares e Silva PL, Bracarense APFRL, Flaiban KKMdC, Akagi CY, et al. Impact of a single oral acute dose of aflatoxin B1 on liver function/cytokines and the lymphoproliferative response in C57Bl/6 mice. Toxins. 2017;9(11):374.
23. Guengerich FP, Johnson WW, Shimada T, Ueng Y-F, Yamazaki H, Langouët S. Activation and detoxication of aflatoxin B1. Mutat Res Fundam Mol Mech. 1998;402(1-2):121-8.
24. Benkerroum N. Chronic and acute toxicities of aflatoxins: Mechanisms of action. Int J Environ Res Public Health. 2020;17(2):423.

25. Xu Q, Shi W, Lv P, Meng W, Mao G, Gong C, et al. Critical role of caveolin-1 in aflatoxin B1-induced hepatotoxicity via the regulation of oxidation and autophagy. Cell Death Dis. 2020;11(1):1-16.
26. de Freitas Souza C, Baldissera MD, Baldisserotto B, Petrolli TG, da Glória EM, Zanette RA, et al. Dietary vegetable choline improves hepatic health of Nile tilapia (Oreochromis niloticus) fed aflatoxin-contaminated diet. Comp Biochem Physiol C Toxicol Pharmacol. 2020;227:108614.
27. Singh C, Prakash C, Mishra P, Tiwari KN, Mishra SK, More RS, et al. Hepatoprotective efficacy of Premna integrifolia L. leaves against aflatoxin B1-induced toxicity in mice. Toxicon. 2019;166:88-100.
28. Xu F, Li Y, Cao Z, Zhang J, Huang W. AFB1-induced mice liver injury involves mitochondrial dysfunction mediated by mitochondrial biogenesis inhibition. Ecotoxicol Environ Saf. 2021;216:112213.
29. Chen F, Feng L, Zheng Y-l, Lu J, Fan S-h, Shan Q, et al. 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) induces mitochondrial dysfunction and related liver injury via eliciting miR-34a-5p-mediated mitophagy impairment. Environ Pollut. 2020;258:113693.
30. Liu X, Taylor SA, Gromer KD, Zhang D, Hubchak SC, LeCuyer BE, et al. Mechanisms of liver injury in high fat sugar diet fed mice that lack hepatocyte X-box binding protein 1. Plos One. 2022;17(1):e0261789.
31. Eiró N, González LO, Cid S, Andicoechea A, Vizoso FJ. Matrix metalloproteases expression in different histological types of colorectal polyps. Rev Esp Enferm Dig. 2017;109(6):414-20.