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Original Research Article

Pathological Effects of Aflatoxin on Hepatic Tissue of Rats, Experimental Study

Heba Saleh Shaheed[1*]

[1]Department of Pathology and Poultry Diseases, Collage of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

***Corresponding Author:** Heba Saleh Shaheed
Department of Pathology and Poultry Diseases, Collage of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

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Abstract: The objective of studying this research is to discuss the pathological influence of aflatoxin on hepatic tissue. Aflatoxin was produced in this work using Aspergillus flavus. The samples were mixed with Potato Dextrose Agar (PDA) and left to incubate at a temperature of 28 ± 2°C for three to five days. We employed the fungal species for further usage once they were incubated. The AF was made via a fermentation process with Aspergillus parasitcus and rice, After the rice had fermented properly, it was steams to destroy the fungus, dried, and pulverised into a powder. The AF concentration in rice residue was determined using a TLC fluorometric densitometer on TLC spots. Ten rats from each of the two experimental groups—"G1 Control" (fed a baseline diet without AF) and "AF" (fed a different diet)—were randomly assigned to participate in the study. Group 2 rats were given 25 µg of AF daily. This study's duration was ninety days. Then, blood specimens were drawn by heart puncture using a syringe to determine serum enzyme levels. The animals were sacrificed in order to get tissue samples as well enzyme levels were assessed in serum specimens that had been centrifuged at 4000 × g for 15 minutes at 4 °C. The levels of AST, ALT, and GGT were determined using a spectrophotometer and a kit that was received from Biolabo. Liver samples were collected from rats after scarification, embedded in paraffin wax, and fixed with 10% neutralised formaldehyde. Hematoxylin and eosin were used for staining. The AF-treated group had substantially high levels of those enzymes compare to the control group ($p < 0.05$). G2 demonstrated that macrophage aggregations in the liver parenchyma constitute granulomatous lesions. Another portion showed hepatocyte apoptosis, which is defined by dense nuclei in the hepatocyte's irregular esonophilic cytoplasm, and a necrotic region with pyknosis or no nuclei at all. A portion of the liver of rats that were treated with aflatoxin showed proliferation of kupffer cells together with a few clusters of mononuclear cells around the major vein. In conclusion, Aflatoxin can affect of liver tissue histology as well as liver enzymes such as ALT, AST as well as GGT.

Keywords: Aflatoxin and hepatic tissue.

INTRODUCTION

Several food items were contaminated with mycotoxins, which are secondary metabolites with severe effects on human and animal health [1]. Agreeing to FAO consider Mycotoxins one of the polluted around a quarter of the world's agricultural raw materials, [2]. This caused health problems and economic losses .In many species, mycotoxins may cause detrimental physiological changes [4]. These low-molecular-weight compounds have the ability to generate both acute and chronic sickness [3].

The five main groups of mycotoxins are as follows: ochratoxins, fumonisins, aflatoxins, zearalenone, and trichothecenes [5]. As per the research conducted by Gong *et al.*, [6], aflatoxins are among the most hazardous mycotoxins as they may induce cancer [7]. No part of the environment is safe from mycotoxins, which pose a major risk to human and animal health and may be found in soil, crops, and foods [8]. In addition to being insoluble in water, aflatoxins (AFTs) are heat resistant, being removed at temperatures as high as 280°C, and solubilizing in methanol and other organic solvents [9]. The fact is that AFTs have a 25% mortality rate [10], and long-term exposure may weaken the

immune system and produce a host of health problems, including death. Growth retardation in children may be caused by a variety of medical conditions, such as cancer, liver damage, and immunological suppression. Out of the eighteen mycotoxins, the four most harmful are aflatoxins G2, G1, B2, and B1. There are a number of aflatoxins, the most toxic of which is AFB1, which is present in many foods such as almonds, rice, and maize [11, 12]. Aspergillus parasiticus produces G2, G1, B2, and B1, whereas Aspergillus flavus produces B2 and B1 [13].

The World Health Organisation has identified AFB1 as one of the naturally occurring compounds with the highest carcinogenic potential. Animal and human studies have shown that it suppresses the immune system, causes birth defects, and harms the liver [14]. International Agency for Research on Cancer has so classified AFB1 as a Group 1 carcinogen. Exposure to AFB1-contaminated substances may cause inflammation, intestinal and liver cell damage in several animals referenced in [15, 16]. It also reduces lymphocyte activation and increases programmed cell death [17]. Meats, eggs, as well as milk that are contaminated with AFB1 may make their way into human diets [18]. Cancers of the gallbladder, colon, lungs, kidneys, liver, and stomach have all been associated with AFB1 [19]. There is evidence that AFT exposure is associated with 4.6-28.1% of hepatocellular carcinoma (HCC) cases [20].

MATERIALS AND METHODS

Aflatoxin was produced by using Aspergillus flavus. The samples were mixed with Potato Dextrose Agar (PDA) and left to incubate at a temperature of 28 ± 2°C for three to five days. We employed the fungal species for further usage once they were incubated.

Aflatoxin Preparation

The AF was made via a fermentation process with Aspergillus parasitcus and rice, following the technique described by [21], with some tweaks made by [22]. After the rice had fermented properly, it was steams to destroy the fungus, dried, and pulverised into a powder. The AF concentration in rice powder was determined using a TLC fluorometric densitometer on TLC spots, following the procedure described by [21]. The AF content of the ground rice powder was 3.66% AFG2, 9.78% AFG1, and 72.51% AFB1. The remaining AF content was 14.05% AFB2. To meet the daily feed requirement of 25 µg of AF per rat, the baseline diet was supplemented with rice powder.

Study Design

Ten rats from each of the two experimental groups—"Control" (fed a baseline diet devoid of AF) and "AF" (fed a different diet)—were randomly assigned to participate in the study. Group B rats were given 25 µg of AF daily. The rats were given diethyl ether to make them unconscious. Then, blood samples were drawn by heart puncture using a syringe to determine serum enzyme levels. The levels of AST, ALT, and GGT were determined using a spectrophotometer and a kit that was received from Biolabo.

The animals were sacrificed in order to get tissue samples as well. Samples were collected from rats after stipulation, embedded in paraffin wax, and fixed with 10% neutralised formaldehyde. Hematoxylin and eosin were used for staining.

Statistical analysis was done by using SPSS version 23.

RESULTS

In Table 1, we can see the combined AST, ALT, and GGT levels of the control and AF-treated groups' livers. The AF-treated group had substantially higher levels of these enzymes compare to the control group ($p < 0.05$).

Figure 1 shows the results of the histological analysis of a liver segment from a control group of rats that were not treated. G2 demonstrated that macrophage aggregations in the liver parenchyma constitute granulomatous lesions (Figure 2). Another portion showed hepatocyte apoptosis, which is defined by dense nuclei in the hepatocyte's irregular esonophilic cytoplasm (Figure 3), and a necrotic region with pyknosis or no nuclei at all (Figure 4). In Figure 5, a portion of the liver of rats that were treated with aflatoxin showed proliferation of kupffer cells together with a few clusters of mononuclear cells around the major vein.

Table 1: Showing Rats serum liver enzyme levels effecting by AF

Enzyme	G1	G2
ALT (U/L)	43.1± 0.97 B	113.6±6.73 A
AST (U/L)	158.28±3.53 B	236±14.89 A
GGT (U/L)	0.26±0.06 B	3.44±0.91 A

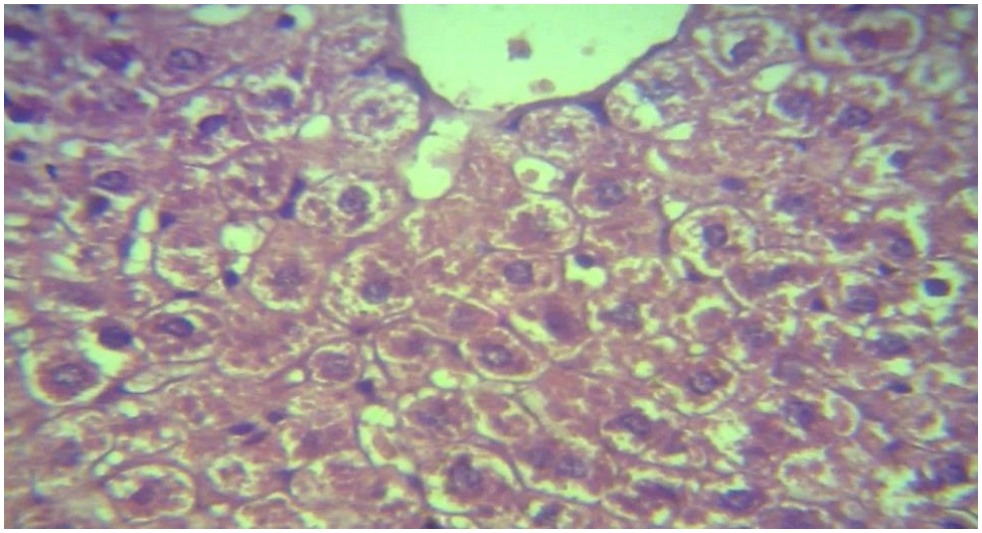

Figure 1: Histopathological section in liver of rat (control) show normal structure (H & E40X)

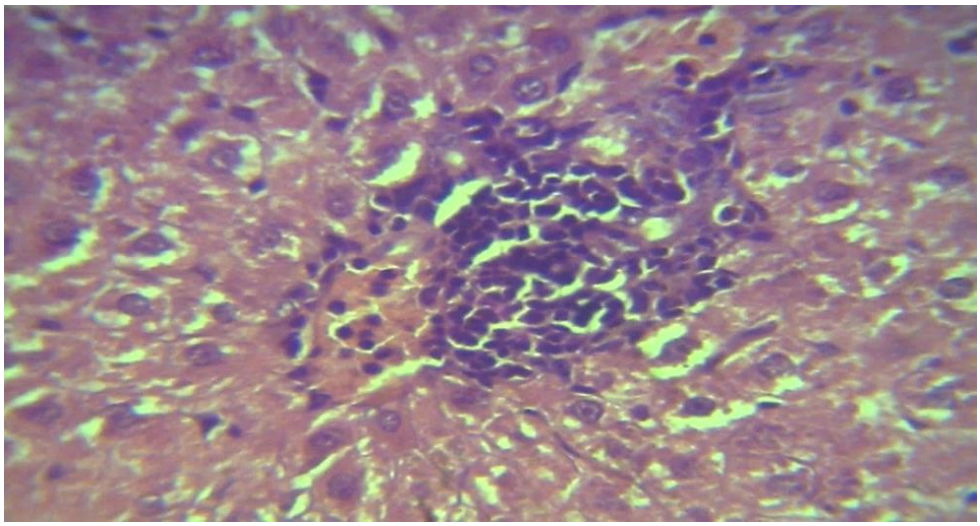

Figure 2: A granulomatous lesion in the liver parenchyma was seen in histopathological sections taken from rats' livers (G2) after they were exposed to AF (H & E 40 X)

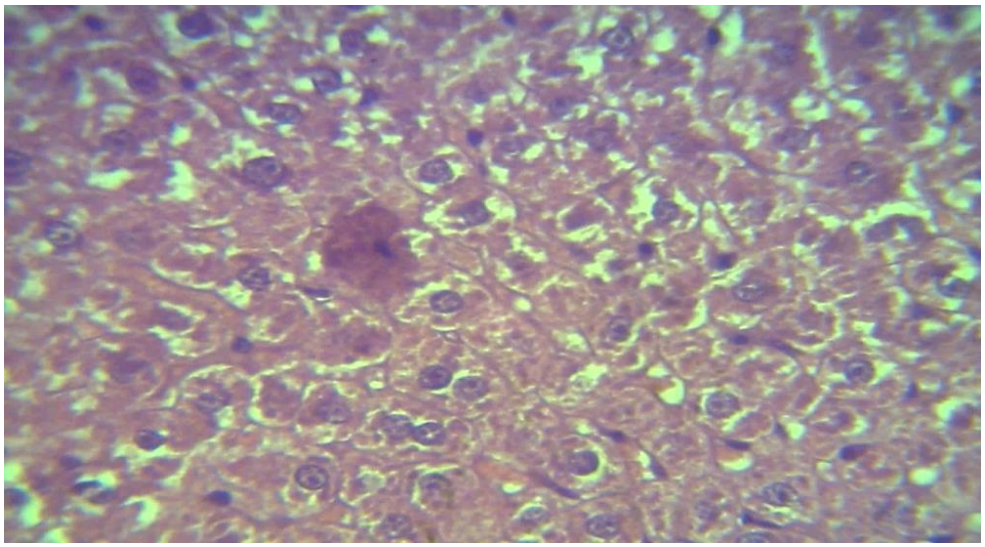

Figure 3: Apoptotic cells are seen in the liver of G2 rats in histopathological sections (H & E 40 X)

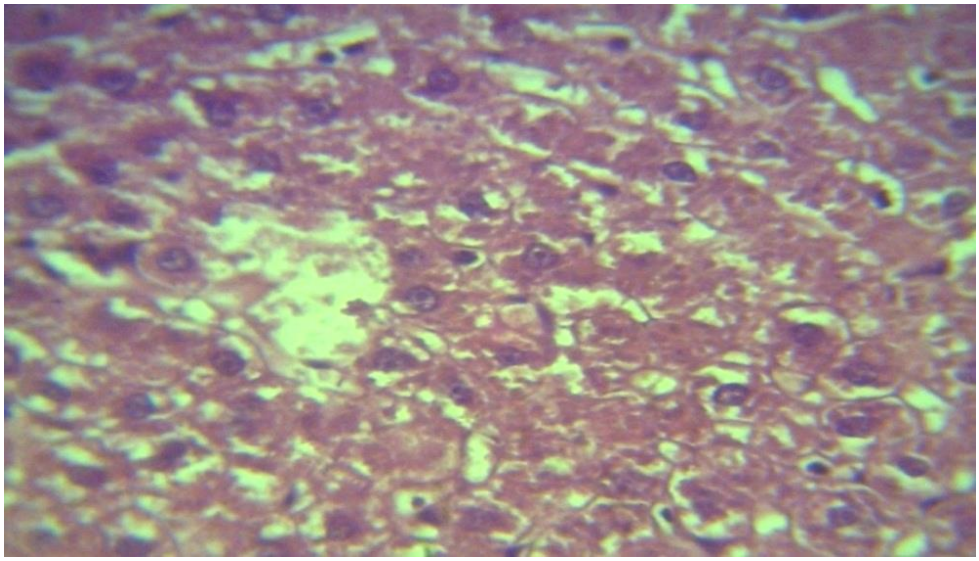

Figure 4: Rat G2 liver histopathology sections reveal that hepatocyte cell nuclei have disappeared (H & E 40 X)

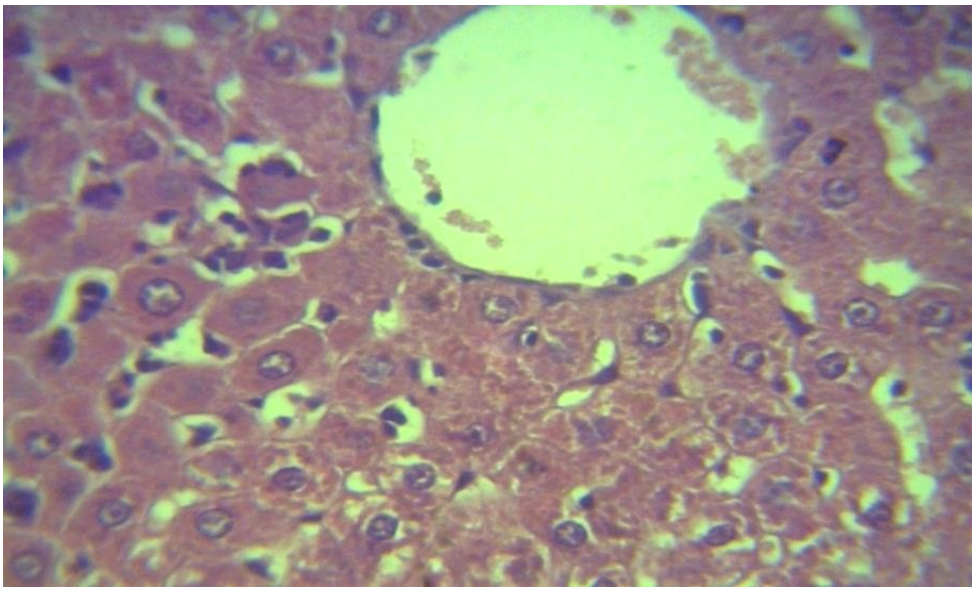

Figure 5: Liver histopathology sections from G2 rats reveal a small number of clumped mononuclear cells around the liver's central vein and parenchyma, as well as the proliferation of kupffer cells (H & E40X)

DISCUSSION

Serum AST, ALT, and GGT values that are consistently rising indicate a decline in liver function. When there is hepatic degradation and necrotic alterations, these enzymes are released into the circulation, which causes serum AST, ALT, and GGT levels to rise [23].

Finding the levels of transaminases is crucial for medical diagnosis. According to [24], hepatobiliary disease and acute hepatic necrosis may be detected by monitoring the performance of ALT and AST. Results showed that ALT, AST, and GGT activities in rat serum were significantly elevated after AFS therapy, and this increase was dosage and time dependent. According to [25], a rise in ALT, AST, and ALP activity indicated acute hepatic necrosis, which is the primary organ that AFS is thought to target. Liu *et al*., [26] also found that rats given AFB1 by gavage had elevated serum AST and ALT activity, which is consistent with our findings. Also, rats given a food polluted with AFS showed a substantial rise in blood AST, ALT, and ALP levels, according to [27]. Similarly, [28] found that after 10 weeks of feeding rabbits diet AFB1, blood AST, ALT, and ALP levels were much higher than normal. In addition, [29] noted that after 72 hours of therapy, AFB1 significantly increased blood ALP, AST, and ALT activity while decreasing it in the liver when administered orally to rats.

This research confirmed previous histological and biochemical evidence of AFB1 toxicity in rats. There is substantial evidence that AF causes liver damage in a number of different animal species [29]. The production of reactive oxygen species (ROS) and the resulting peroxidative damage is the primary process by which

aflatoxin causes hepatotoxicity [30]. Research has shown that AFB1 may compromise cell membrane integrity by promoting lipid peroxidation in cells via A2 induction [31]. ROS production [32], lipid peroxidation [33], and 8-hydroxydeoxyguanosine [34] may all be accelerated by AFB1. In addition to forming AFB1-DNA adducts, AFB1's capacity to generate oxidative damage to cells and DNA may play a significant role in AFB1 carcinogenicity [34], especially in light of ROS's involvement in chemically induced carcinogenesis. There is strong evidence that the formation of aflatoxin epoxide derivatives, which are dependent on hepatic microsomal cytochrome P-450 in particular, is pivotal to the development of AFB1 toxicity and carcinogenic activity [35]. In addition, These epoxide metabolites have been shown to disrupt cell integrity by binding to macromolecules such as nuclear proteins and nucleic acids, inhibiting the production of enzymes and proteins [36]. Hepatocytes in the periacinar regions of the liver have the largest concentration of these enzyme systems, which are known as hepatic microsomal cytochrome P-450 [37]. Our investigation found that the periacinar and intermediate areas of liver lobules in the treatment groups, particularly the AF-treated group, exhibited histological alterations such as hazy edoema, hydropic degeneration, and localised necrosis. Some studies have shown that aflatoxicosis liver lesions are mostly located in the periacinar region, while others have shown that they are intermediately located [38, 39]. There are three grades of hepatocellular deterioration in aflatoxicosis: 1) Lesions that are mild in nature are marked by hepatocellular swelling as a result of hydropic degeneration and fatty changes; 2) Lesions that are moderate in severity are identified by diffused-severe hepatocellular swelling, cytoplasmic pallor, and rupture; and 3) Lesions that are severe in severity are characterised by clear hepatocellular swelling limited to the periacinar and midzonal areas.

CONCLUSION

Aflatoxin can effect of liver tissue histology as well as liver enzymes such as ALT, AST as well as GGT.

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